Optimization of Flow Cytometric Analysis for Regulatory and Inflammatory Eosinophils

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Introduction

The eosinophil is a granulocytic innate immune cell that has been studied since the 18th century, however, scientists still frequently debate the roles of eosinophils in the body. Evolutionarily, the eosinophil developed to combat parasitic infections but is also recognized as a significant contributor to the pathogenesis of allergic diseases such as allergic asthma and eosinophilic esophagitis. More recently, published data suggest that eosinophils are also important for the development and homeostasis of the lung, where two groups of eosinophils have been described: Homeostatic/Resident eosinophils (R-Eos) and Inflammatory eosinophils (i-Eos).

The goal of this project is to optimize a flow cytometric antibody panel that will allow us to examine rEos and iEos during lung development as well as in a model of allergic asthma. The ability to analyze these populations by flow cytometry will provide a tool that we can use to examine the mechanisms by which regulatory and inflammatory eosinophils arise in the lung. The specific markers that are used to distinguish eosinophils include viability, CD45, Siglec-F, CD11c, CD11b. The markers CD101 and Ly6-G will be used to distinguish between the regulatory and inflammatory eosinophils as published data describe regulatory eosinophils as both CD101-Lo and Ly6G-Lo while inflammatory eosinophils are CD101-Hi and Ly6G-Hi.

Methods

Mice

Male and female C57Bl/6 mice (PN10-6 months old) were bred and housed in a specific pathogen-free facility at Cincinnati Children's Hospital according to protocols approved by the Institutional Animal Care and Use Committee.

Allergen treatment protocols

Mice were intratracheally treated with 40ul of PBS or 10ug of allergen in 40ul PBS for at least 3 exposures to induce an allergic asthma response.

Isolation of lung cells

Mice were euthanized with sodium pentobarbital and lungs were extracted, minced, and digested in a 6-well tissue plate with a 70um cell filter and Liberase TL. A single cell suspension was obtained for analysis by flow cytometry.

Flow cytometry stain protocols and analysis

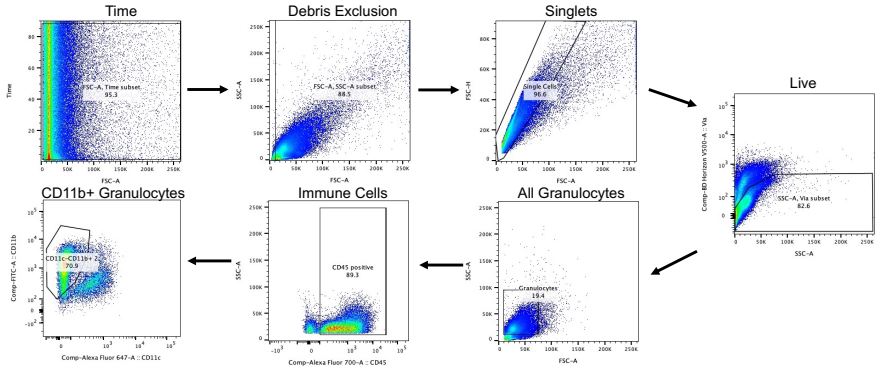
Staining was performed the day of cell isolation using the following antibodies (clones): CD101-PE (Moushi101), Ly6G-PE (IA8), CD11b-FITC (M1170), CD11c-AF647 (N418), CD45-AF700n(30-F11), SiglecF-BV421 (E5-2440), and Viability-v500. Data analysis was performed using FlowJo software and utilizing single stained cells, beads, and Fluorescence Minus One (FMO) cell controls

Results

Table 1. Cell surface markers and the expression levels on cell populations in the lung.

Cells	CD101	Siglec-F	CD11b	CD11c	Ly6G	CD45
rEosinophils	-/Lo	+	+	+	-/Lo	+
iEosinophils	Hi	+	+	+	Hi	+
Endothelial	-	-	-	-	-	-
Neutrophils	-/+	-	-	+	+	+
Epithelial	-	-	-	-	-	-
Alveolar Macrophages	-	-	-	+	-	+
T cells	-	-	-	-	-	+
B cells	-	-	-	-	-	+

Figure 1. Gating strategy to examine the eosinophil compartment.



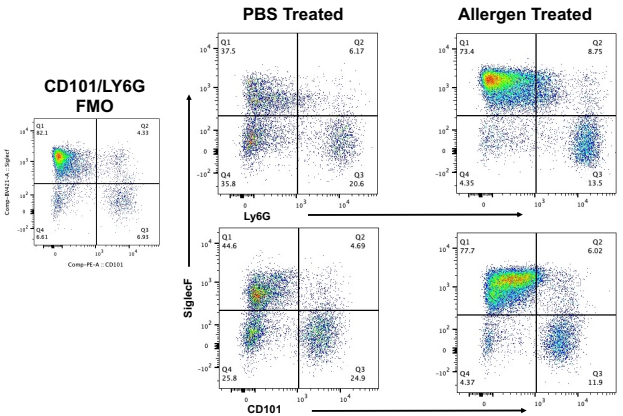


Figure 2. A comparison of Ly6G and CD101 expression levels on eosinophil populations. Total lung cells from PBS and allergen treated mice were isolated and stained used the markers listed in Table 1. The CD101/Ly6G-PE FMO is shown on the left. Ly6G and CD101 expression levels are compared on live CD45+ SiglecF+ CD11b+ CD11c- populations. The pseudocolor plots shown are from one representative mouse of 3-6 mice per group.

Conclusion

- Mouse regulatory and inflammatory eosinophils are CD45+ SiglecF+ CD11b+ and CD11c-
- Regulatory eosinophils, predominantly found in mice treated with PBS, are CD101 negative/Lo
- Regulatory eosinophils, predominantly found in mice treated with PBS, are Ly6G negative/Lo
- Inflammatory eosinophils, elevated in allergen-treated mice, are CD101 Hi
- Inflammatory eosinophils, elevated found in mice treated with PBS, are Ly6G Hi
- Both Ly6G and CD101 reveal a shift towards increased inflammatory eosinophils (elevated CD101 or Ly6G expression) in allergen treated mice

Future Directions

- Create a panel that combines CD101 and Ly6G on separate colors to examine levels of both receptors on the same cells
- Sort regulatory and inflammatory eosinophils using CD101 and Ly6G Lo/Hi expression levels to obtain populations that may be further examined morphologically
- Examine eosinophil populations in bronchoalveolar lavage fluid and total lung cells during lung development
- Examine eosinophil populations in bronchoalveolar lavage fluid and total lung cells in a mouse model of allergic asthma

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