Fast and Efficient Enrichment of Functional ILC2 From Human Whole Blood

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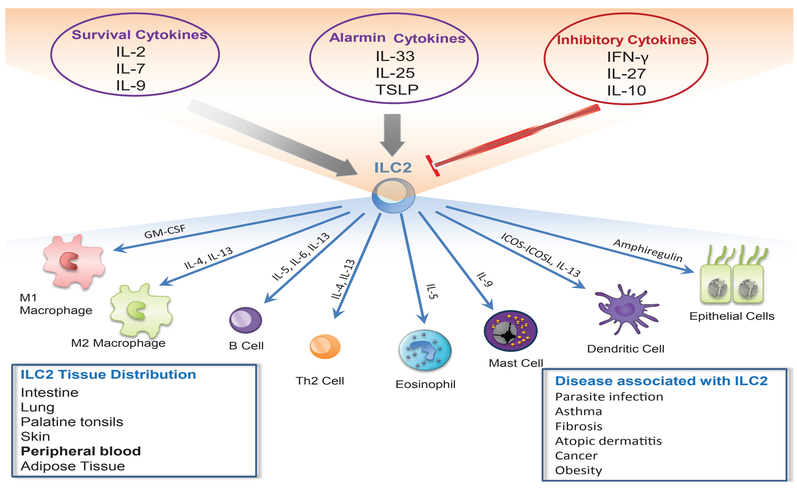
Introduction

Figure 1. Group-2 Innate Lymphoid Cells (ILC2)



- Share characteristics with T cells and produce T helper cell associated cytokines
- Lack rearranged antigen-specific receptors
- Play a critical role in the pathology of Th-2 diseases such as allergies, asthma, and helminth infections, as well as metabolic diseases

ILC2 are difficult to study due to paucity, heterogeneity and similarity to other ILCs

- ILC2 are widely distributed throughout the body but they are extremely rare. The frequency of ILC2 in blood is approximately 0.01% of human leukocytes
- They do not express surface markers of mature lymphocytes and myeloid markers (Lin^-). No unique markers specific for individual ILC populations are known
- Currently, multicolor flow cytometric cell sorting is the only method to isolate ILC2. However, sorting of ILC2 is time-consuming, expensive and often results in low purities and recoveries due to the scarcity of these cells in primary tissues

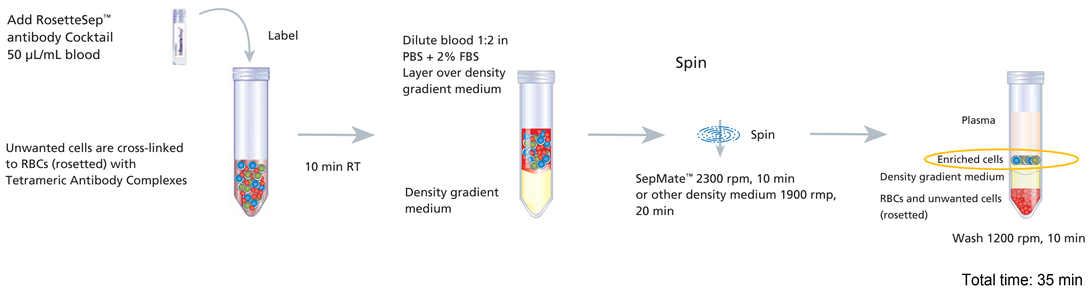
GOAL: To develop a fast and simple procedure to pre-enrich ILC2 from whole blood by negative selection prior to cell sorting

Materials and Methods

Samples

ILC2 were isolated either by density gradient centrifugation using Lymphoprep™ (Cat# 07801) and SepMate™ (Cat# 15450) (unenriched) or using RosetteSep™ (enriched) as shown in Figure 2.

Figure 2. Negative Selection of Human ILC2 Enrichment From Whole Blood Using RosetteSep™



Cell Sorting

60 mL of unenriched or RosetteSep™-enriched samples were FACS sorted. Sorting time, purity and recovery were recorded and compared between the 2 approaches. Cells were cultured, stimulated and IL-13 production was analyzed by ELISA.

Assessment of ILC2

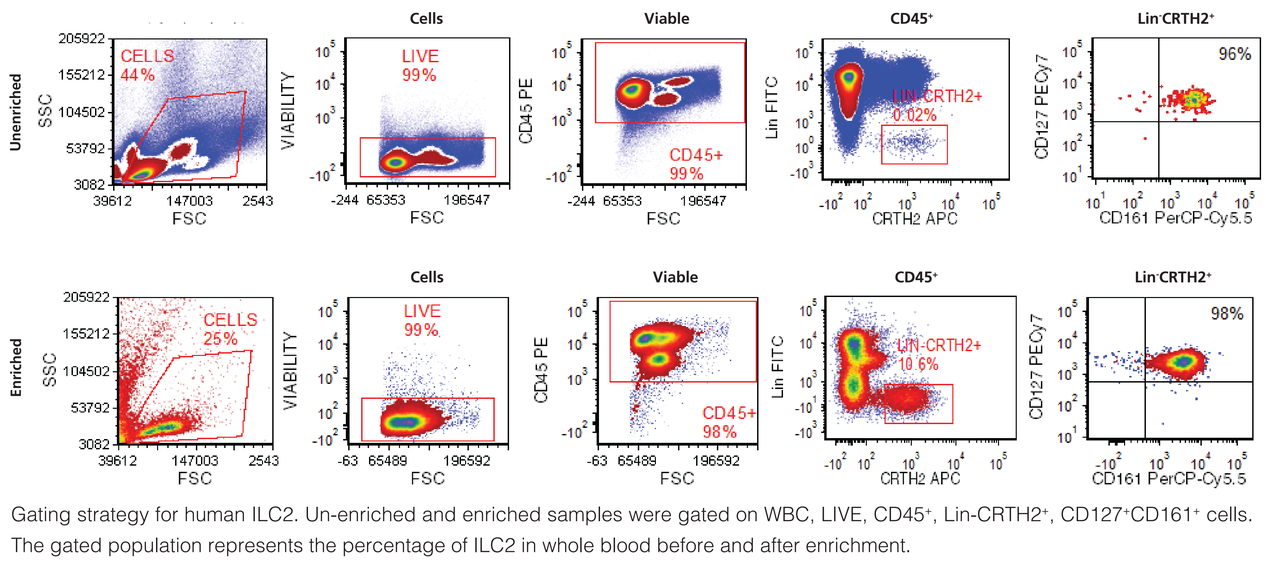
ILC2 were assessed using the following gating scheme: WBC → LIVE (Viable) → $CD45^+$ → LIN^-CRTH2^+ → $CD127^+CD161^+$
Antibodies used in the lineage cocktail include, CD1a, CD3, CD11c, CD14, CD19, CD34, CD123, TCRαβ, TCRγδ, BDCA2, FcεR1

Conclusions

- ILC2 frequency was between 0.002 to 0.07% after density gradient centrifugation alone (n=38)
- ILC2 were enriched to 0.44 – 53% with RosetteSep™ (n=38)
- FACS sorting from enriched samples was faster (n=4, $p<0.05$ paired t test), and yielded higher ILC2 purity than FACS sorting from unenriched samples
- When stimulated in culture, sorted ILC2 from both enriched and unenriched samples secreted similar high levels of IL-13 as assessed by ELISA
- Enrichment of ILC2 prior to FACS sorting increased purity, shortened sorting time, and maintained ILC2 functionality

Results

Figure 3. Percentage of ILC2 in whole blood, before and after enrichment



Gating strategy for human ILC2. Un-enriched and enriched samples were gated on WBC, LIVE, $CD45^+$, Lin^-CRTH2^+, $CD127^+CD161^+$ cells. The gated population represents the percentage of ILC2 in whole blood before and after enrichment.

Figure 4. Percentage of ILC2, fold enrichment and number of ILC2 in blood before and after enrichment (n = 38)

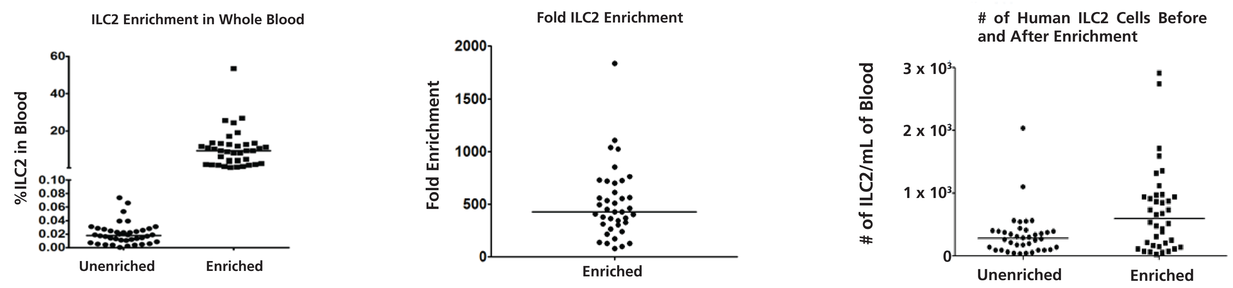


Table 1. Enrichment saves time and provides better ILC2 purity

Scenario 1: Focused on Recovery

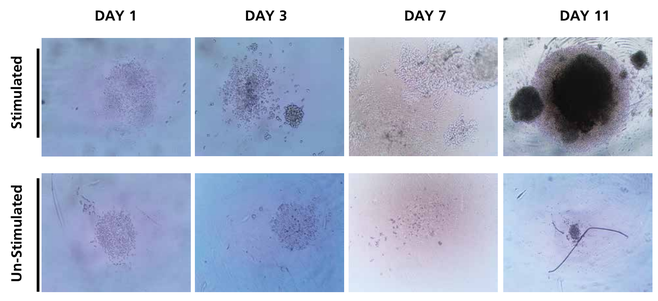
Scenario 1	Unenriched Sample	RosetteSep™ Enriched Sample
Volume of whole blood processed and time	60 mL ammonium chloride lysis, 30 min	60 mL RosetteSep™ Enriched, 30 min
% of ILC2	0.01%	3%
Total Number of Cells Stained	130 x 10^6	0.42 x 10^6
First Sort: Time	87 min	8 min
Volume Sorted	60 mL (all processed)	All obtained after enrichment
Number of Cells	17489	10831
Purity	67%*	99%
Calculated # ILC2s post-sort	11738	10831

*Second sort needed for good purity

Scenario 2: Focused on Purity

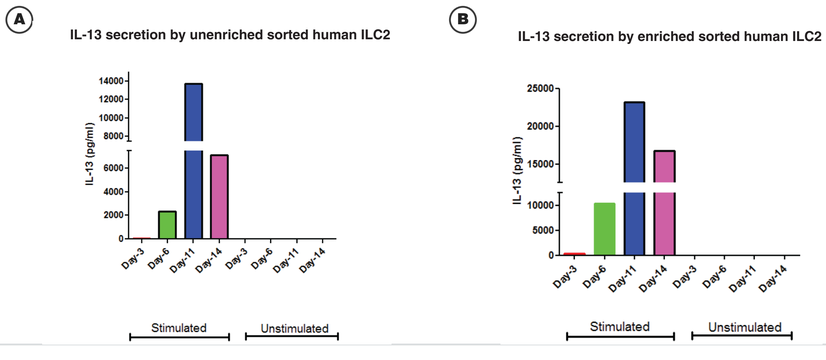
Scenario 2	Unenriched Sample	RosetteSep™-Enriched Sample
Volume of whole blood processed and time	60 mL (2X ammonium chloride lysis, 30 min)	60 mL RosetteSep™-Enriched, 30 min
% ILC2	0.05%	37%
Total Number of Cells Stained	200 x 10^6	0.39 x 10^6
First Sort: Time	60 min	5 min
Volume Sorted	10% of stained sample	All
Number of ILC2	3231	21305
Purity	46.75%	98.43%
Second Sort Time	8 min	No second sort
Purity	97.16%	98.43%
Number of ILC2	1719	21305
Calculated time to sort entire sample and # ILC obrained	~ 11 hours ~ 17000 ILC2	5 min 21305

Figure 5. RosetteSep™-enriched and sorted ILC2 are able to proliferate



RosetteSep™-enriched cells were sorted and stimulated using a cytokine cocktail. Pictures show proliferation at different time points. Non-stimulated cells served as controls.

Figure 6. RosetteSep™-enriched ILC2s are functional and secrete IL-13



PBMCs were obtained by density gradient centrifugation (A) or RosetteSep™enrichment (B) prior to sorting for ILC2. Sorted cells were stimulated at different days and the supernatant analyzed for IL-13 by ELISA.