Pre-enrichment of Human ILCs Improves Sorting Efficiency While Maintaining Functionality

Yanet Valdez[1], Stephen K. Kyei[1], Grace F.T. Poon[1], Cassielle Dent[1], Carrie E. Peters[1], Steven M. Woodside[1], Allen C. Eaves[1,2] and Terry E Thomas[1]

[1] STEMCELL Technologies Inc., Vancouver BC, Canada [2] Terry Fox Laboratory, BC Cancer Agency, Vancouver BC, Canada yanet.valdez@stemcell.com

Introduction

MAIN CHARACTERISTICS OF "HELPER-LIKE" INNATE LYMPHOID CELLS (ILCs)

- Produce T helper cell-associated cytokines
- Lack rearranged antigen-specific receptors
- Do not express surface markers of mature lymphocytes and myeloid markers (Lin-)
- Play critical roles in the pathology of many diseases

CHALLENGES WORKING WITH ILCs

- Extremely rare (approximately 0.06% of human leukocytes in blood)
- Lack unique markers specific for individual ILC populations
- Flow cytometric cell sorting is the only method to isolate ILCs, which is time-consuming, expensive, and often results in low purities and recoveries

GOAL: To develop a fast and simple procedure to enrich ILCs from peripheral blood by negative selection prior to cell sorting

Methods

Enrichment Strategies

Figure 1. Human ILC2 enrichment from whole blood by negative selection using RosetteSep™.

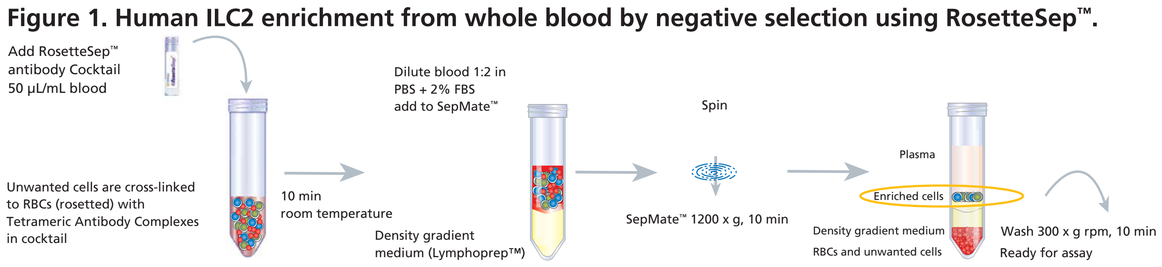
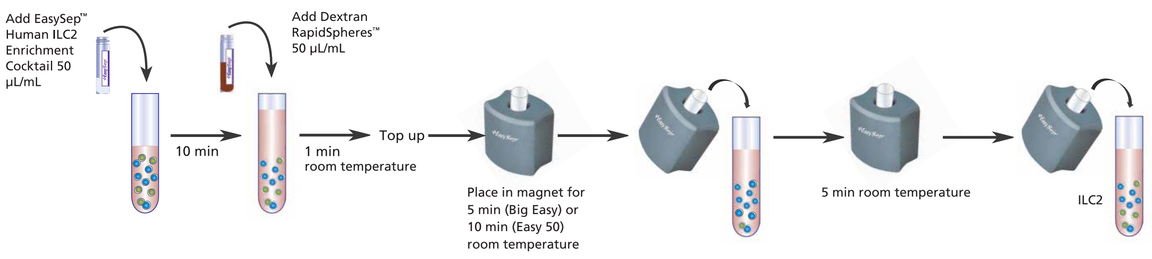


Figure 2. Human ILC enrichment from leukapheresis by negative selection using EasySep™.



Assessment of ILCs by Flow Cytometry

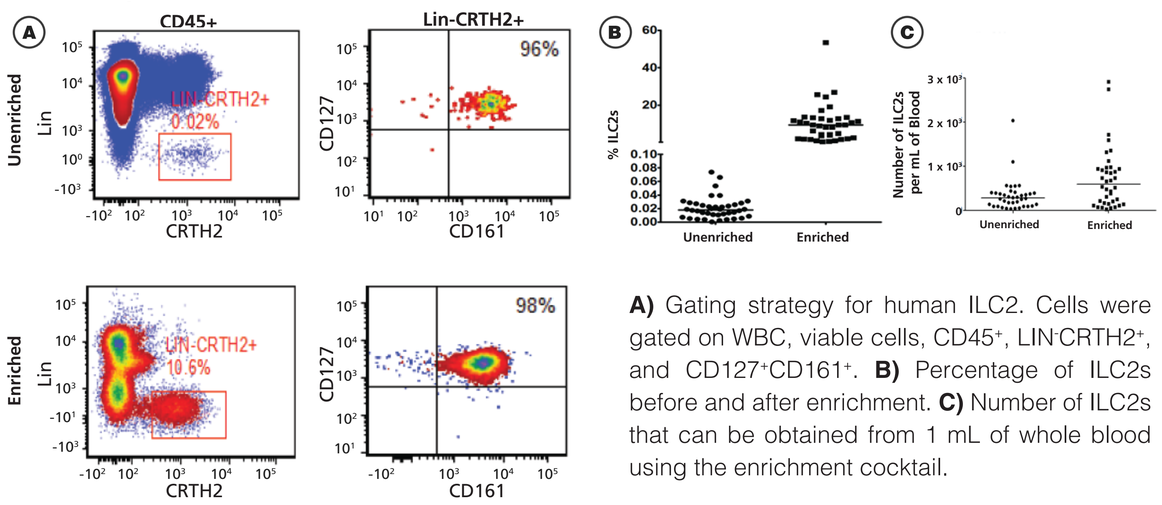
ILCs were identified as CD45$^+$, Lineage-negative (CD1a, CD3, CD11c, CD14, CD19, CD34, CD123, TCRαβ, TCRγδ, BDCA2, FcεR1, CD94, CD4, CD16), and CD127$^+$. ILC1 was defined as Lin$^-$CD127$^+$CRTH2$^-$CD117$^-$. ILC2 was defined as Lin$^-$CD127$^+$CRTH2$^+$CD117$^{+/-}$. ILC3 was defined as Lin$^-$CD127$^+$CRTH2$^-$CD117$^+$.

Sorting of ILC2s by FACS and Functional Analysis

The efficiency of ILC sorting from unenriched and enriched whole blood and leukapheresis samples was compared. Sort time and ILC purity were analyzed. Sorted ILCs were stimulated *in vitro*, and their ability to produce cytokines was measured by ELISA.

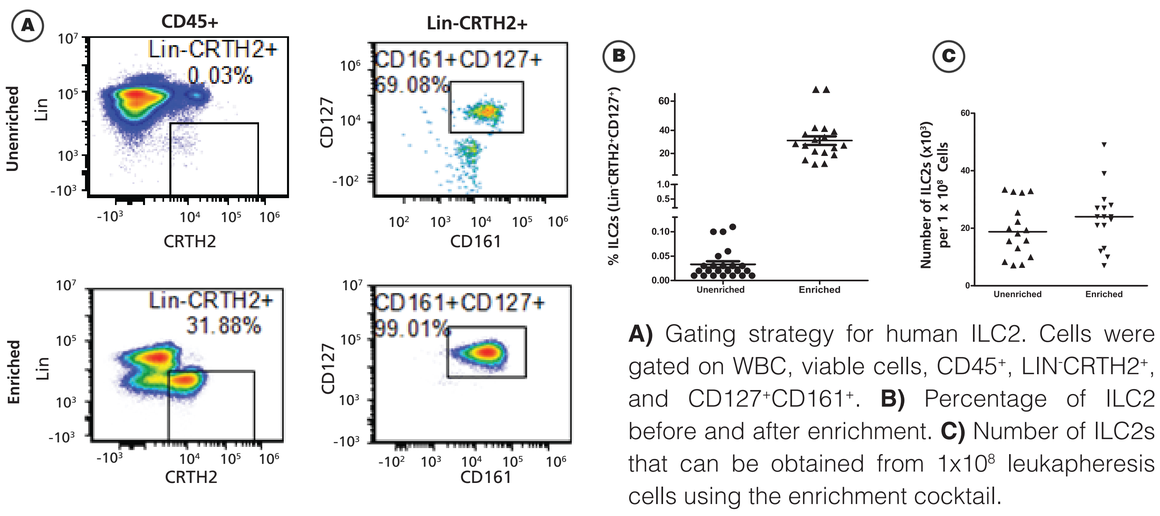
Results

Figure 3. Percentage and number of ILC2s before and after enrichment from blood using RosetteSep™.



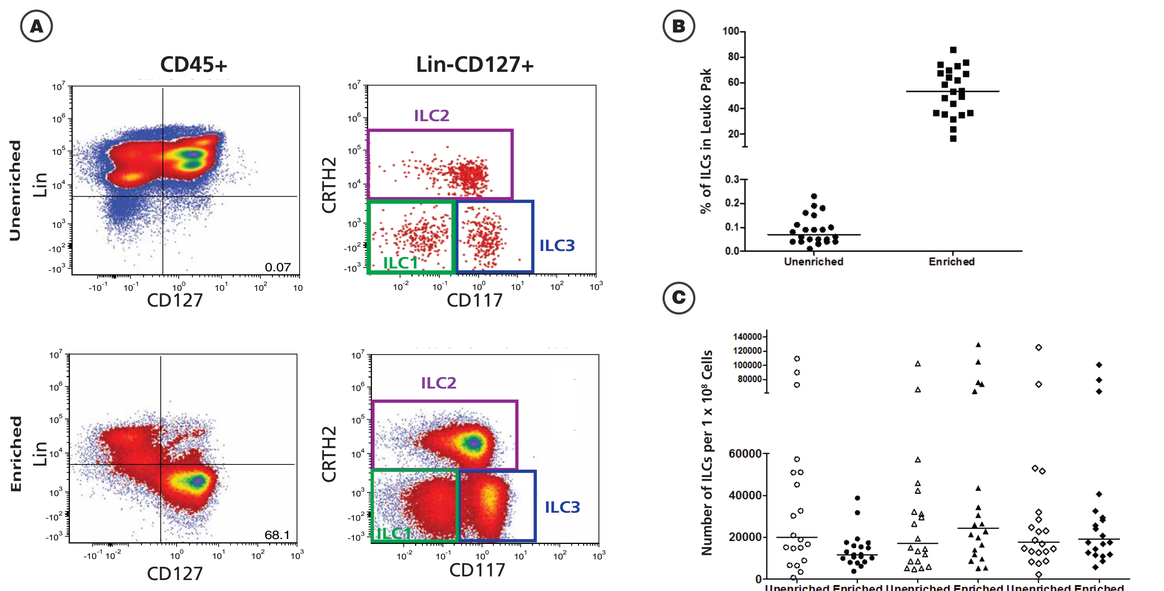
A) Gating strategy for human ILC2. Cells were gated on WBC, viable cells, CD45$^+$, LIN$^-$CRTH2$^+$, and CD127$^+$CD161$^+$. **B)** Percentage of ILC2s before and after enrichment. **C)** Number of ILC2s that can be obtained from 1 mL of whole blood using the enrichment cocktail.

Figure 4. Percentage and number of ILC2 before and after enrichment from leukapheresis using EasySep™.



A) Gating strategy for human ILC2. Cells were gated on WBC, viable cells, CD45$^+$, LIN$^-$CRTH2$^+$, and CD127$^+$CD161$^+$. **B)** Percentage of ILC2 before and after enrichment. **C)** Number of ILC2s that can be obtained from 1x10^8 leukapheresis cells using the enrichment cocktail.

Figure 5. Percentage and number of Pan ILCs before and after enrichment using EasySep™ negative selection.

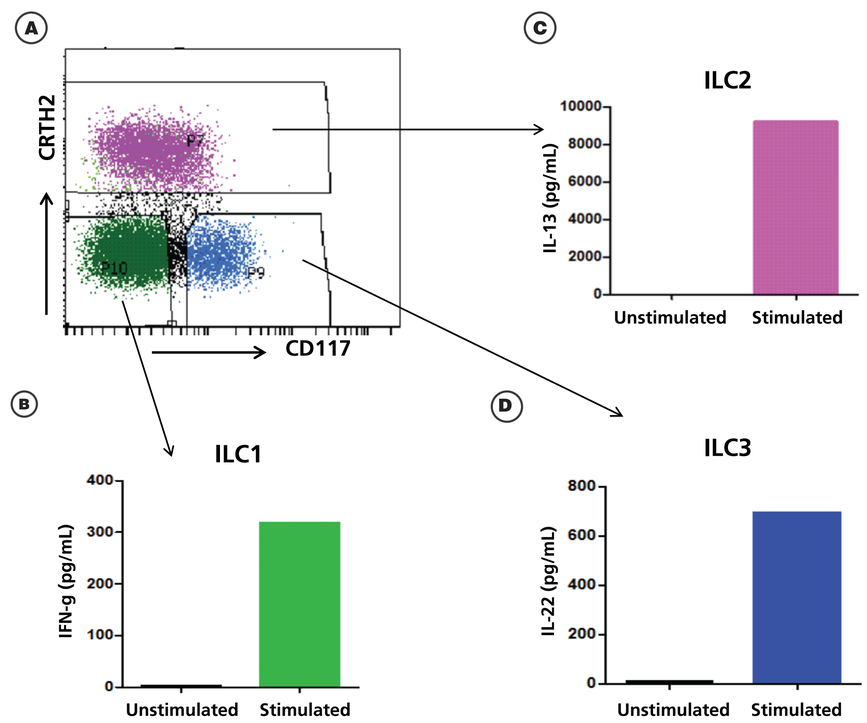


A) Cells were gated on WBC, viable cells, CD45$^+$, LIN$^-$, and CD127$^+$. **B)** Percentage of Pan ILCs before and after enrichment. **C)** Number of ILCs that can be obtained from 1x10^8 leukapheresis cells using the enrichment cocktail.

Table 1. EasySep™ Pan ILC enrichment improves sorting time and purity.

	Enriched	Unenriched	Hypothetical (If Sorting Entire Unenriched Sample)
Number of cells before enrichment	2 x 10^9	7.5 x 10^7	2 x 10^9
Number of cells after enrichment	4.1 x 10^6	N/A	N/A
Purity before sort	27%	0.1%	0.1%
Number of ILCs after sort #1	3.68 x 10^5	3.54 x 10^4	1.37 x 10^6
Purity after sort #1	99%	76%	76%
Recovery after sort #1	33%	35%	35%
Purity after sort #2	N/A	97%	97%
Sample preparation time	0.5 hr	0.3 hr	0.3 hr
Total sort time	0.2 hr	2 hr	103 hr

Figure 6. EasySep™-enriched ILCs are functional.



Sorted enriched ILCs were cultured with or without stimuli, and the supernatants were analyzed for cytokine secretion by ELISA. **A)** Cell sorting gates for ILC subsets. **B to D)** Cytokine secretion by stimulated ILCs.

Conclusions

- ILC2 frequency was between 0.004 to 0.07% after density gradient centrifugation (n = 38) in whole blood and 0.001 to 0.16% in leukapheresis (n =12)
- ILC2s were enriched from whole blood to 0.44 - 53% after density gradient separation with RosetteSep™ (n = 38). Using EasySep™, ILC2s were enriched from leukapheresis to 13 - 78%
- Pan ILCs were enriched from leukapheresis from 0.01 - 0.23% to 17 - 86% (n = 22)
- Sorted ILCs from enriched samples were stimulated in culture. They secreted high levels of IFN-g (ILC1), IL-13 (ILC2), and IL-22 (ILC3) as assessed by ELISA, indicating that these cells are functional
- Enrichment of ILCs prior to sorting increases purity, shortens sorting time, and maintains ILC functionality