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Highly pure type 2 innate lymphoid cells from human blood

Isolation of highly pure type 2 innate lymphoid cells from human blood

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Introduction

The percentage of human type 2 innate lymphoid cells (ILC2s) found in peripheral blood mononuclear cells (PBMCs) is around 0.05%. As such it is often useful to isolate a highly pure cell population to facilitate further study. Human ILC2s are defined as lineage (CD2, CD3, CD14, CD16, CD19, CD56, CD235a, CD123)–negative, CD127$^+$, CD161$^+$, and CRTH2$^+$ lymphocytes[1]. Lineage-positive cells can be removed using a MicroBead Kit for lineage cell depletion from Miltenyi Biotec, which increases the purity of ILC2s to around 10%. To improve ILC2 purity, we have developed a protocol using MACS® Technology that combines lineage depletion with enrichment of CRTH2$^+$ cells by positive selection. The optimized process allowed us to achieve cell purities of greater than 90%.

Materials and methods

PBMCs were obtained from 50 mL heparinized human blood using density gradient centrifugation as previously described[2]. ILC2s were then enriched using a two-step process as illustrated in figure 1. Initially, the lineage-positive, i.e., CD2$^+$, CD3$^+$, CD11b$^+$ CD14$^+$, CD15$^+$, CD16$^+$, CD19$^+$, CD56$^+$, CD123$^+$, and CD235a (Glycophorin A)$^+$ cells were depleted using the Lineage Cell Depletion Kit, human and two LS Columns (Miltenyi Biotec).

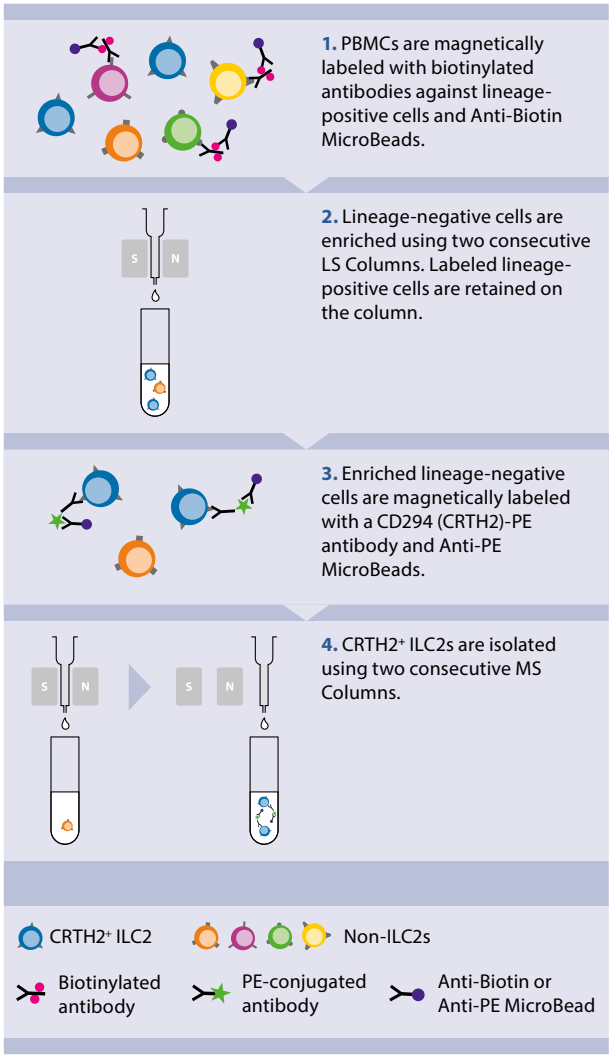

Figure 1: Schematic outlining the protocol for the isolation of CRTH2$^+$ ILCs from PBMCs. Initially the lineage-positive cells are depleted using two LS Columns. The CRTH2$^+$ cells are then isolated from the lineage-negative cell fraction by positive selection using two consecutive MS columns. The steps involving the second LS and MS Columns for lineage cell depletion and positive selection of ILC2s, respectively, have been omitted from the schematic.

The manufacturer's protocol was followed, with the exception that after obtaining the lineage-negative cells from the first LS Column, the cells were centrifuged at 300×g for 10 min, resuspended in 500 µL buffer, and applied to a second column. The negative cells eluted from this second column were taken forward for CRTH2$^+$ cell enrichment using the CD294 (CRTH2) MicroBead Kit and two MS Columns. Again the manufacturer's instructions were followed. However, labeling with the CD294 (CRTH2)-PE antibody was performed at room temperature for 20 minutes. ILC2 purity was assessed in the PBMCs, the lineage-depleted fraction and enriched CRTH2$^+$ cells by flow cytometry (fig. 2). This optimized protocol provided the basis for the development of a new kit, containing all reagents required for the effective isolation of CRTH2$^+$ ILCs, now available from Miltenyi Biotec.

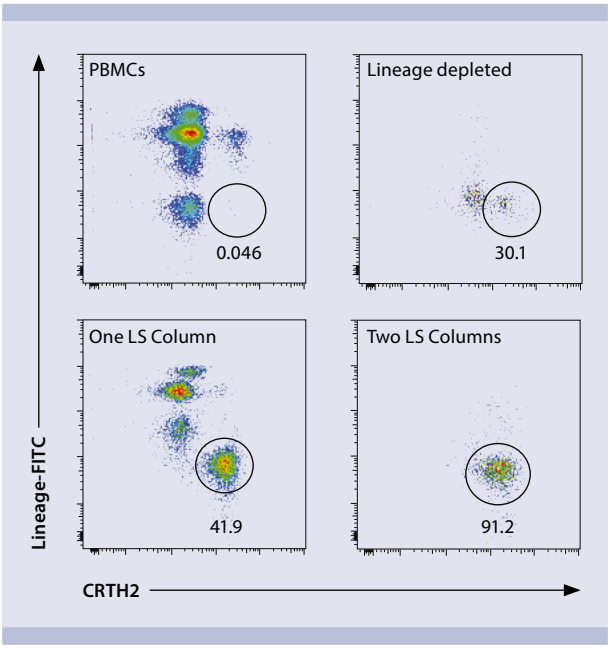

Figure 2: Flow cytometry analysis of PBMCs, lineage-depleted cell fraction, and the enriched CRTH2$^+$ cells (bottom panel) following the use of one or two LS Columns. Cells were stained with a cocktail of FITC-conjugated antibodies against lineage markers and a fluorochrome CRTH2-specific antibody. Gates were drawn to determine percentages of ILC2s (lineage negative, CRTH2$^+$) present in each fraction. The use of two LS Columns for lineage cell depletion increased the purity of CRTH2$^+$ cells significantly.

Results

Using the Lineage Cell Depletion Kit increased the percentage of ILC2s to around 10% on average. The use of the kit for CRTH2$^+$ cell enrichment as directed by the manufacturer's protocol (all antibody labeling performed at 4 °C) was initially unsuccessful, however, by performing the CRTH2$^+$ cell labeling step at room temperature we were able to obtain a significant enrichment of ILC2s (around 40%). To further improve the purity of the ILC2s we introduced a second LS Column in the lineage depletion step to remove more of the contaminating cells, which enabled us to achieve ILC2 purities of 90% or more (figs. 2 and 3). The process allowed us to isolate 3326 ILC2s per 10^8 PBMCs on average (fig. 3). These ILC2s remained viable, lineage-negative producers of type 2 cytokines even after several weeks of *in vitro* culture.

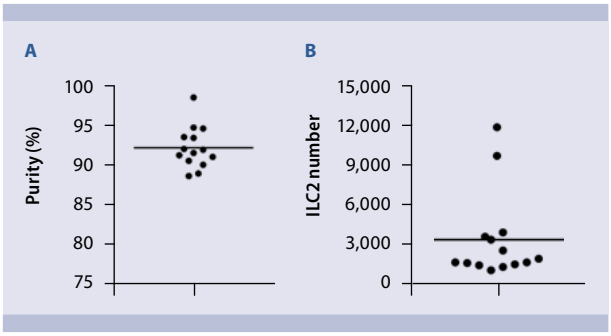

Figure 3: Purity (A) and yield (B) of isolated CRTH2$^+$ cells. Data were obtained by flow cytometry in 14 separate experiments. The lines denote the mean values. The ILC2 yield is specified as the number of isolated ILC2s per 10^8 PBMCs.

Conclusion

The combined use of two MicroBead kits from Miltenyi Biotec allowed us to develop and optimize a protocol to isolate lineage-negative, CRTH2$^+$ ILC2s from human blood. We have reliably achieved purities of around 90% using this procedure. The isolated cells are suitable for *in vitro* culture and further study. A complete kit for the isolation of CRTH2$^+$ cells based on this protocol is now available from Miltenyi Biotec.

References

1. Mjösberg, J.M. *et al.* (2011) Human IL-25– and IL-33–responsive type 2 innate lymphoid cells are defined by expression of CRTH2 and CD161. Nat. Immunol. 12: 1055–1062.
2. Jackson, D.J. *et al.* (2014) IL-33–dependent type 2 inflammation during rhinovirus-induced asthma exacerbations *in vivo*. Am. J. Respir. Crit. Care Med. 190: 1373–1382.

MACS Product	Order no.
ILC2 Isolation Kit, human	130-114-825
LS Columns	130-042-401
MS Columns	130-042-201

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