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Specialized Modalities

Cryopreservation of Peripheral Blood Mononuclear Cells

Does not alter immune cell phenotype

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This study examines the variation of immune cell types in isolated PBMCs from different donors through the use of an immune phenotyping assay. We analyzed the impact of cryopreservation by comparing the immune phenotypes of PBMCs isolated from fresh and cryopreserved leukopaks. Our findings indicate that immune profile of the various cell type sub-populations was donor-dependent and did not change upon successive cryopreservation

Introduction

Peripheral blood mononuclear cells (PBMCs) consisting of lymphocytes, dendritic cells, and monocytes play an important role in both the innate and adaptive immune systems and are critical in protecting the body from viral, bacterial, and parasitic infection.

PBMCs are increasingly being used in the drug development journey of biological therapeutics. Although ideally, researchers may like to use freshly isolated PBMCs, it is not always feasible to isolate the cells and generate experimental data immediately. Typically these cells are isolated from fresh leukopaks and cryopreserved for future use.

To assess the impact of cropreservation on the various sub-populations of immune cells, we compared the percentage of lymphocytes, dendritic cells and monocytes within PBMC populations from both fresh leukopaks and leukopaks that had been previously frozen (cryopreserved leukopaks).

In this tech note, we studied the prevalence of immune cell types in isolated PBMCs through the use of an immune phenotyping assay. We analyzed the impact of cryopreservation by comparing the immune phenotypes of PBMCs isolated from fresh leukopaks within 24 hours of collection and cryopreserved with the phenotype of PBMCs isolated from frozen leukopaks from the same donor. We demonstrated that the immune profile of the various cell type sub-populations was donor-dependent and did not change upon successive cryopreservation.

These findings enable researchers to select from an increased repertoire of donor material and can be confident that PBMCs isolated from cryopreserved leukopaks will be equivalent in profile to fresh isolations.

Materials

- Normal Mouse IgG 5mg (Sigma P/N M7894)
- CD45 FITC (BD Biosciences P/N 347463)
- CD45 V450 (BD Biosciences P/N 642275)
- CD4 APC (BD Biosciences P/N 340443)
- CD8 FITC (BD Bioscience P/N 347313)
- CD3 PE (BD Biosciences P/N 347347)
- CD56 APC (BD Biosciences P/N 341025)
- CD45 PerCP-Cy 5.5 (BD Biosciences P/N 340953)
- CD45 APC (BD Biosciences P/N 340943)
- CD11c PE (BD Biosciences P/N 347637)
- CD19 PerCP-Cy5.5 (BD Biosciences P/N 340951)
- CD14 FITC (BD Biosciences P/N 347493)
- Live/Dead Fixable Near-IR Dead Cell Stain Kit (Invitrogen P/N L100119)
- FACS buffer

Methods

PBMCs from four donors were isolated either from fresh leukopaks (PBMC isolation occurred within 24 hours of leukopak collection from donors) or from cryopreserved leukopaks (leukopaks from the same donors that were cryopreserved prior to PBMC isolation). Isolated PBMCs from both leukopak types were subsequently cryopreserved again. After a 2-week cryopreservation, cells were thawed and assessed based on the protocol below:

Immune phenotyping

1. PBMCs were thawed at 37° C for 2 minutes, then centrifuged at 300 xg for 10 minutes
2. After centrifugation, cells were counted using Trypan Blue and resuspended at 10×10^6 cells per mL FACS buffer
3. FACS buffer is prepared by adding 15 mL FBS and 2 mL of 25% Sodium azide solution to 500 mL of PBS (Ca^{++} and Mg^{++} free)
4. Cells were then incubated with Normal Mouse IgG in FACS Buffer for 30 minutes on ice in the dark
5. Cells were washed with PBS and incubated with antibodies following the vendor's recommended protocol for 30 minutes on ice in the dark
   a. $CD45^+$ Lymphocyte marker
   b. $CD3^+$ T Cells
   c. $CD4^+$ T helper Cells

d. $CD8^+$ Cytotoxic T Cells
e. $CD19^+$ B Cells
f. $CD56^+$ Natural Killer Cells
g. $CD14^+/CD11c^+$ Monocytes/Dendritic Cells

6. After incubation, cells were washed with PBS and stained with live/dead stain following the vendor's recommended protocol for 30 minutes on ice in the dark
7. Cells were washed with PBS and fixed in 2% PFA then analyzed on the BD Canto II flow cytometer

Results

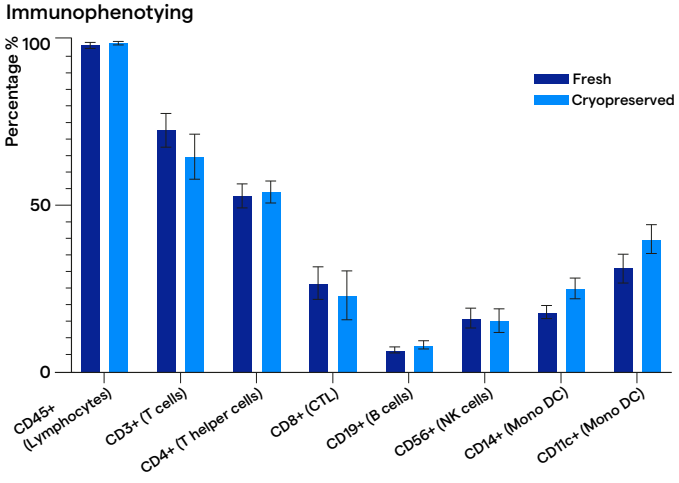

Figure 1.
Percentage of individual immune cells analyzed immediately out of cryopreservation via FACS. PBMCs were isolated either from fresh or cryopreserved leukopak. Dark purple bars represent immune cell populations in PBMCs isolated from fresh leukopak. Light purple bars represent immune cell populations in PBMCs isolated from cryopreserved leukopak. All populations were gated on live, single cell population for flow cytometry analysis. Data is the combined average of the 4 donors tested and represented as the mean ±SEM. Populations were analyzed by two-way ANOVA.

Cryopreserved leukopaks have similar cell populations as fresh leukopaks

To determine general percentages of different lymphocytes, dendritic, and monocyte cell types, PBMCs were isolated and cryopreserved from leukopaks of 4 donors that were processed in the following manner: Leukopak samples were collected from each donor and split into two halves where one half of the sample was tested as fresh leukopak for PBMCs to be isolated within 24 hours of leukopak collection, while the other half was cryopreserved post-collection, then subsequently completely thawed for PBMC isolation. Each donor was analyzed for various markers via FACS. Results demonstrate that cryopreserving leukopaks prior to PBMC isolation does not significantly impact the percentage of specific immune cell populations (Figure 1). However, data does indicate that, as expected, donors differ in the percentages of specific sub-populations present (Figure 2). Specifically, donor 1 and donor 4 showed lower percentages of T cells, but higher percentage of monocytes and dendritic cells in PBMCs isolated from cryopreserved leukopak compared to fresh. This difference in cell populations may be attributed to better preservation of monocyte and dendritic cell populations, but less superior T cells preservation in cryopreserved compared to fresh leukopak for donors 1 and 4. Meanwhile, donors 2 and 3 showed similar presence of all assessed immune cells in PBMCs isolated from both fresh and cryopreserved leukopaks (Figure 2). Overall, both types of leukopaks showed the presence of the same immune cell populations across different donors (Figure 2), though the pencentage of these cell types in PBMCs may differ due to donor-to-donor variability, as expected.

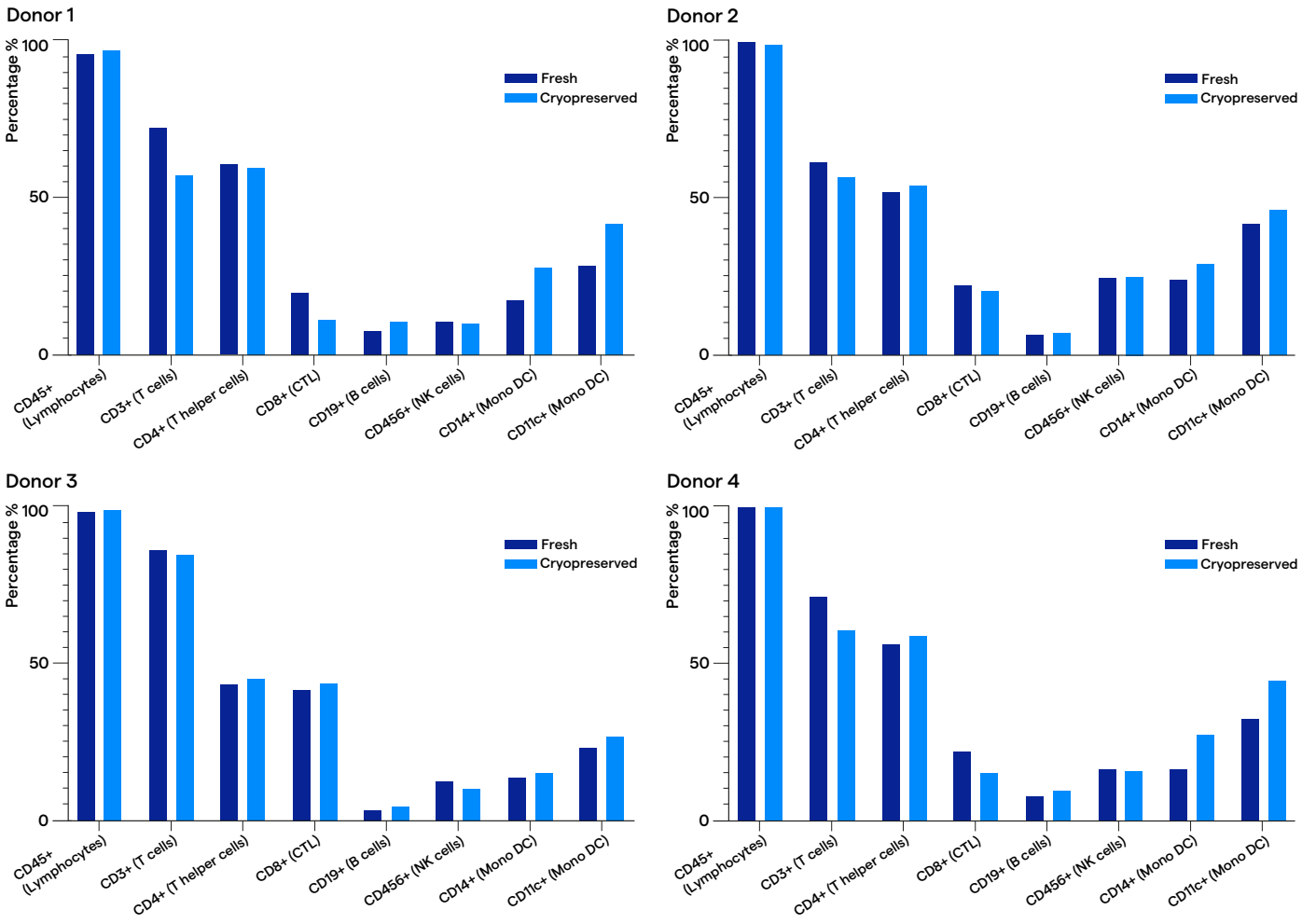

Figure 2.
Percentage of individual immune cells analyzed immediately out of cryopreservation via FACS for the four donors assessed. PBMCs were isolated either from fresh or cryopreserved leukopak. Dark purple bars represent immune cell populations in PBMCs isolated from fresh leukopak. Light purple bars represent immune cell populations in PBMCs isolated from cryopreserved leukopak. All populations were gated on live, single cell population for flow cytometry analysis.

Summary

As donor variation becomes more important to researchers, Lonza is now offering cryopreserved and fresh leukopaks, in addition to isolated PBMCs. Ready to use solutions such as Lonza PBMCs increases the repertiore of convenient tools that researchers can select from to find donors that meet their specific study needs.

To assess the impact of cryopreservation on immune cell phenotype, we compared the profile of various immune cell populations in PBMCs isolated from fresh and cryopreserved leukopaks from the four donors. This analysis allowed us to see whether the proportion of specific immune subtypes remained equivalent between PBMCs isolated from these two groups.

This data demonstrated that percentage of specific cell types was dependent on the donor, and these differences in immune profile was maintained whether the PBMCs were isolated from fresh or frozen leukopaks (Figure 2). Thus, immune phenotyping data can enable researchers to select PBMC lots more suited for their individual research needs. Further, cryopreserving the leukopaks prior to PBMC isolation did not alter the percentage of cell populations, indicating the utility of previously isolated and frozen PBMCs as a viable and convenient alternative to in house isolations (Figure 2). With an increasing need for PBMCs, researchers can choose from cryopreserved, fresh leukopaks, or previously isolated cryopreserved PBMCs for those interested in a ready-to-use product. These options increases researcher's abilities to obtain donors that meet specific study needs.

hPBMC Specification

Species	Human			
Cell Source	Peripheral Blood			
Donor	Normal, various donor profiles avaliable			
Cell Type	Cryopreserved Mononuclear Cells			
Part Code	CC-2702	CC-2703	CC-2704	CC-2705
Cell Number	50 M	100 M	10 M	25 M

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