Human platelet lysate as a media supplement for *ex vivo* expansion of immune cells

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BACKGROUND

Adoptive immunotherapy with *ex vivo* derived T lymphocytes (T cells) and natural killer cells (NK cells) has emerged as an effective therapeutic strategy to treat various types of cancer and autoimmune diseases. Adoptive cellular therapy involves the *ex vivo* expansion of lymphocytes under cGMP regulations. The use of fetal bovine serum (FBS) as a supplement for cell culture carries a risk of pathogen transmission as well as xenoimmunization against bovine antigens. Human AB serum (ABS), another option for T cells and NK cells, has supply limitations and therefore may not be sufficient to meet the expected demand for immunotherapies. Human platelet lysate (hPL) obtained from transfusable donated platelets is widely recognized as a valuable alternative to both FBS and ABS for manufacturing clinical cell product.

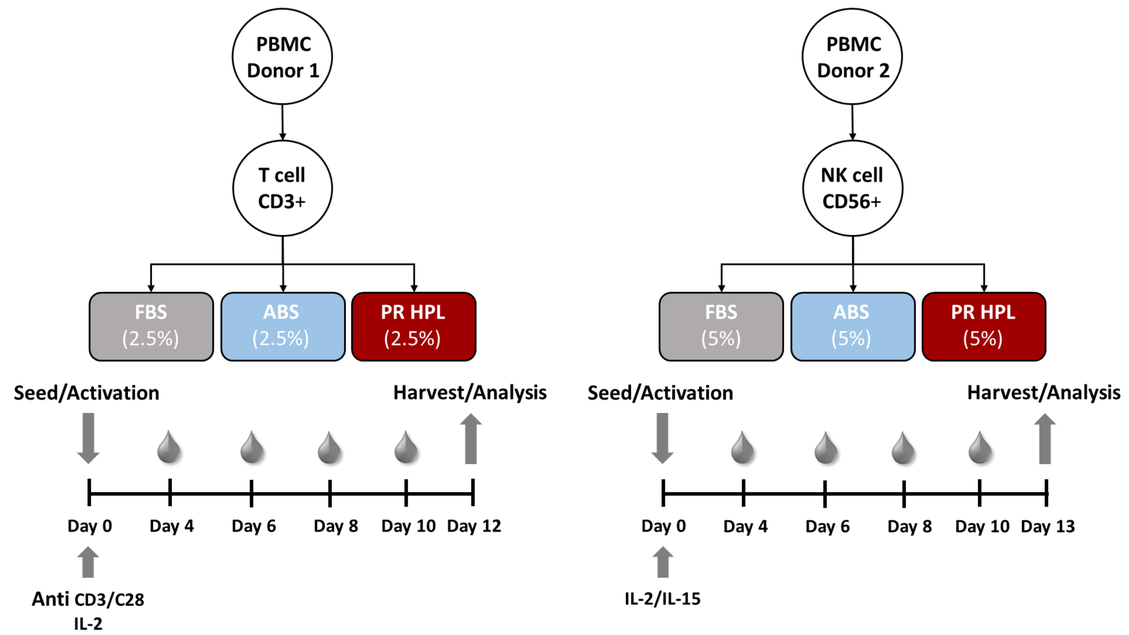
Despite the low risk with platelet units that are screened and tested to the same stringent criteria in place for transfusable blood, the transmission of infectious agents remains a consideration for human derived ancillary products, including ABS and hPL. Pooling platelet concentrates improves hPL consistency, but increases the statistical risk of viral contamination. In order to mitigate the viral transmission risk, we have produced a pathogen-reduced HPL (PR HPL) that has shown a high level of viral reduction (enveloped and non-enveloped) while preserving functionality as a cell culture supplement.

PURPOSE

The goal of the present study was to explore the potential use of PR HPL as an alternative to FBS and ABS for the *ex vivo* expansion of human T cells and NK cells.

METHODS

Figure 1. Experimental design



T cell isolation and expansion

- CD3+ cells were enriched from cryopreserved peripheral blood mononuclear cells (PBMCs) by negative immunomagnetic separation using EasySep™ Human T cell Isolation Kit (STEMCELL™ Technologies).
- At day 0, T cells were seeded in a 24-well plate at density of $0.5x10^6$ cells/mL and activated with ImmunoCult™ Human CD3/CD28 T Cell Activator (STEMCELL Technologies) and 100 U/mL of human recombinant IL-2 (STEMCELL Technologies).
- Cells were maintained in AIM V Medium (Thermo Fisher Scientific) containing 2.5% of different media supplements: heat-inactivated FBS (Sigma-Aldrich®), heat-inactivated human ABS (Sigma-Aldrich), and PR HPL in presence of IL-2 at 37°C and 5% CO_2.
- Cells were counted at days 4, 6, 8, 10, and 12 and cell density adjusted to $1x10^6$ cell/mL with fresh medium.
- Cells were analyzed at day 12 of culture by flow cytometry using the following monoclonal antibodies: CD3 (clone REA613), CD4 (clone M-T466), CD8 (clone REA734) from Miltenyi Biotec; CD62L (clone DREG-56), CCR7 (clone 047H7), CD45RO (clone UCHL1), and CD45RA (clone HI100) from BioLegend®. Samples were analyzed on a FACSCalibur™ flow cytometer (BD Biosciences).

NK cell isolation and expansion

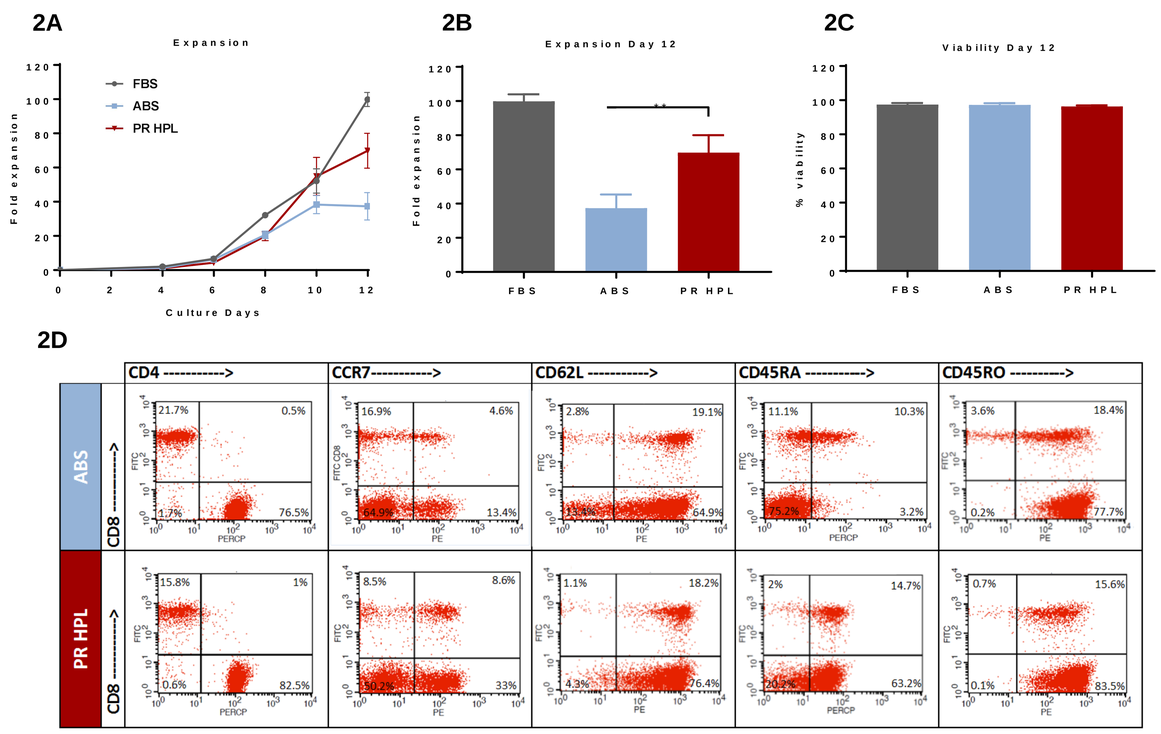
- CD56+ cells were purified from cryopreserved PBMCs by positive immunomagnetic separation using EasySep™ NK Cell Isolation Kit (STEMCELL Technologies).
- At day 0, NK cells were seeded in a 24-well plate at a density of $0.5x10^6$ cells/mL and activated with 500 U/mL of human recombinant IL-2 and 15 ng/mL of IL-15 (STEMCELL Technologies).
- Cells were maintained in AIM V Medium containing 5% of different media supplements: heat-inactivated FBS, heat-inactivated ABS, and PR HPL in presence of IL-2 and IL-15 at 37°C and 5% CO_2.
- Cells were counted at days 4, 6, 8, 10, and 13 and cell density adjusted to $1x10^6$ cell/mL with fresh media.
- Cells were analyzed at day 0 and day 13 of culture by flow cytometry using the following monoclonal antibodies: CD3 (clone REA613, Miltenyi Biotec), CD56 (clone 5.1H11, BioLegend), CD16 (clone 3G8, BioLegend). Samples were analyzed on a BD Accuri™ C6 flow cytometer (BD Biosciences).

Statistical analysis

- Data are presented as mean ± standard deviation of three technical replicates. Statistical analyses between groups were carried out using one-way ANOVA. $p<0.05$ was considered to indicate a statistically significant difference (*=$p<0.05$, **= $p<0.001$, NS = not significant).

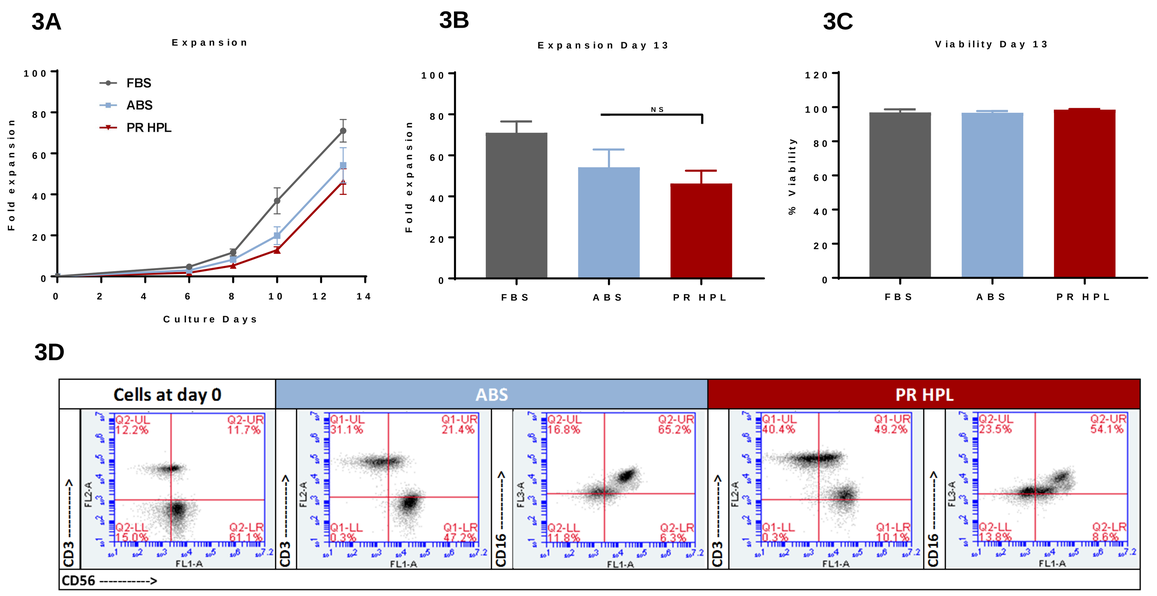
RESULTS

Figure 2. CD3+ cell expansion and characterization



2A. Expansion kinetics of CD3+ cells. **2B.** Total fold expansion at day 12. **2C.** Viability at day 12. **2D.** Representative phenotypic analysis of CD3+ cells by flow cytometry. The expression of CD4, CD8, CCR7, CD62L, CD45RA, and CD45RO markers was assayed on day 12 for both ABS and PR HPL expanded cells.

Figure 3. CD56+ cell expansion and characterization



3A. Expansion kinetics of CD56+ cells. **3B.** Total fold expansion at day 13. **3C.** Viability at day 13. **3D.** Representative phenotypic analysis of CD56+ cells by flow cytometry. The expression of CD56, CD3, CD16 markers was assayed on day 0 and day 13 for both ABS and PR HPL expanded cells.

CONCLUSION

- After 12 days *ex vivo* expansion of human CD3+ T cells, PR HPL showed increased expansion compared to ABS. Cells expanded with PR HPL showed increased expression of CCR7 and CD45RA markers compared to ABS, suggesting an expansion of the naïve and central memory T cell populations.
- In studies using human CD56+ cells, PR HPL showed an expansion rate at 13 days comparable to ABS. Cells grown in PR HPL showed a global increase in CD3+ expression compared to ABS (89.6% vs 52%), again indicating that CD3+ cells thrive in PR HPL. These studies also showed that PR HPL supported the expansion of the CD56+/CD3+ population of NK T cells.
- Overall the data support the use of PR HPL as an ethically sourced, xeno-free alternative to FBS and human ABS for *ex vivo* expansion of human T lymphocytes and natural killer cells.

CONTACT INFORMATION

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