A new platelet lysate alternative to serum for ex vivo transduction and expansion of human T cells

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Background
Adaptive immunotherapy with T lymphocytes (T cells) modified ex vivo has emerged as a promising therapeutic strategy to treat various cancer and autoimmune diseases. T cells engineered to express chimeric antigen receptors (CARs) have shown high rates of clinical responses in hematologic malignancies and even early indications of clinical activity in solid tumors. The manufacture of CAR T cell therapies typically begins with autologous collection of mononuclear cells via leukapheresis, followed by enrichment of the T cell population, and finally genetic modification with viral vectors and ex vivo expansion. The use of fetal bovine serum (FBS) as a supplement for T cell culture carries a risk of pathogen transmission as well as auto-immunization against bovine antigens. Human AB serum, another cell culture option for T cells, has supply limitations and therefore may not be sufficient to meet the expected demand for immunotherapies. Human platelet lysate (HPL) obtained from transfusable donor platelets is widely recognized as a valuable alternative to both FBS and human AB serum for production of clinical cellular therapies.

Purpose
The goal of the present study was to explore the feasibility of using a new pathogen-reduced human platelet lysate (PR HPL) in ex vivo manipulations of T cells, including transduction and expansion. The intent was to determine if PR HPL would be a suitable alternative to human AB serum for T cell culture, and if so, to compare it to human AB serum with respect to transduction efficiency and T cell expansion.

Methods
• CMV-GFP lentiviral particles were supplied by the Indiana University Vector Production Facility (Dr. Ken Cornetta)
• ImmunocultTM CD3/CD28 T cell Activator, STEMCELL Technologies
• IL2 (100 U/mL working concentration), STEMCELL Technologies
• Supplements (at concentrations indicated in figures):
  - Human Peripheral Blood Pan-T cells, STEMCELL™ Technologies
  - Human AB serum (heat inactivated), Sigma H5667
  - FBS, Gibco™ 16000-044
  - IL2 (100 U/mL, working concentration), STEMCELL Technologies
  - Immuncult™ CD3/CD28 T Cell Activator, STEMCELL Technologies
  - CMV-GFP lentiviral particles were supplied by the Indiana University Vector Production Facility (Dr. Ken Cornetta)
• Four-color flow cytometry was performed on an Accuri C6 or Accuri C6 Plus (BD).
• For flow cytometry data, immunostaining was performed with the following antibody compensation beads (ThermoFisher): Fluorescein minus one (“FMO”) controls and unstained cells were used to define gates.

Key Reagents
• Human Peripheral Blood Pan-T cells, STEMCELL™ Technologies
• Basal medium: CTS®™ V™, Thermofisher Scientific
• Supplements (at concentrations indicated in figures): PR HPL, Cook Regentec(R) (R&D produced) human AB serum (heat inactivated), Sigma H5667
• FBS, Gibco™ 16000-044
• IL2 (100 U/mL, working concentration), STEMCELL Technologies
• Immuncult™ CD3/CD28 T Cell Activator, STEMCELL Technologies
• CMV-GFP lentiviral particles were supplied by the Indiana University Vector Production Facility (Dr. Ken Cornetta)

Transduction Study
- Transduction: Analysis ten and seven days after transduction
- Expansion Study: Replicating Experiments
  - 14-15 days after ex vivo expansion in PR HPL

Figure 1. Efficiency of lentiviral transduction of CMV-GFP transgene into T cells in presence of human AB serum or PR HPL.
(A) Percent GFP positive two days after transduction. Each graph shows mean and standard deviation for technical replicates (n=3) from one donor. Four donors are shown.
(B) Percent GFP positive one week after transduction, a timepoint when indirect effects of each supplement on culturing would be expected to be minimal. (B) Percent GFP positive one week after transduction.
(C) Mean fluorescence intensity (‘brightness’) one week after transduction. Each graph shows mean and standard deviation for technical replicates (n=3) from one donor. Four donors are shown.

Figure 2. Effect of supplement concentration on CMV-GFP lentiviral transduction. Percent GFP positive two days after transduction. Each graph shows mean and standard deviation for technical replicates (n=3) from one donor. Four donors are shown.

Figure 3. Efficiency of lentiviral transduction of EF1A:KUB-GFP and UBC:GFP transgenes into T cells in presence of various supplements. Percent GFP positive (left panels) or mean fluorescence intensity (right panels) one week after transduction. Each graph shows mean and standard deviation for technical replicates (n=3) from one donor. Note that the quantity of each of the two lentiviruses/transgenes (EF1A:KUB-GFP and UBC:GFP) applied to the cells (multiplicity of infection) was not standardized for each donor and the data are not meant to be compared across transgenes. Single donor tested per transgene.

Figure 4. T cell expansion in various cell supplements. (A) Cells were thawed and activated in media containing 5% of each supplement. Cells were counted and volume measured on the indicated days to obtain total cell counts. (B) Four donors were tested and each plot is shown individually as results varied by donor. (B) The total cell expansion observed on day 13 or day 14 was normalized to the first count obtained on day 4. No significant difference was observed between PR HPL and AB serum (Student’s t-test).

Figure 5. CD4 and CD8 analysis by flow cytometry
(A) Dot plots showing gating on representative data from one donor. Cells were thawed and activated in media containing 5% PR HPL. (B) Histogram summarizing the mean and standard deviation obtained from four donors analyzed on day 14 after thawing and expansion in media containing 5% of each supplement.

Conclusions
• Cells cultured in PR HPL have characteristics that may be ideal for CAR T cell therapy:
  - Transduction efficiency similar to other supplements
  - Expansion similar to 5% AB serum
  - Maintenance of preferred 4060/4048 CD4/CD8 ratio
  - High proportion Td7/Td8 (about 3.5x more Td7 than AB serum), Td7 phenotype may be associated with improved long-term tumor killing (Becker et al., Curr Opin Hematol. 2015;4:113-9. 2015).
  - Certain transgenes yielded more robust expression when cells were cultured in PR HPL. This could translate to reduced volumes of lentivirus needed to achieve similar functional effects.

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Using PR HPL to support the ex vivo phase of the CAR T gene/cell therapy process

Markers used to assess T cell differentiation

PR HPL supports less differentiated state

Figure 4. CCDC31 and CD44RO analysis by flow cytometry
(A) Flow cytometry plots showing the phenotype of CD4+ gated T cells cultured with PR HPL versus those cultured with AB serum, where the PR HPL has less undifferentiated TEM (CD45RO+CCR7-) cells. All four donors tested showed this shift in phenotype. A similar effect is seen with CD8+ gated T cells (not shown). (B) Summary data showing the proportion of TEM, TCM, TN TCM, TN, or TEM (left) or CD4+ T cells (right). The mean of four experiments are represented in the bar graphs.

Figure 5. CD4 and CD8 analysis by flow cytometry
Cells were thawed and cultured in the indicated supplements for 14-15 days prior to analysis. (A) Representative histograms showing distribution of CD69, fluorescence from one donor. Left shows the population gated on CD4+ cells and right is gated on CD8+ cells. (B) Summary data showing mean and standard deviation and individual replicates of patient CD69+ from three donors. Statistical analyses between groups were carried out using one-way ANOVA. (*p<0.05, **p<0.01)

Figure 7. CD69 analysis by flow cytometry
(A) Dot plots showing gating on representative data from one donor. Cells were thawed and activated in media containing 5% PR HPL.
(B) Histogram summarizing the mean and standard deviation obtained from four donors analyzed on day 14 after thawing and expansion in media containing 5% of each supplement.