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

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Background

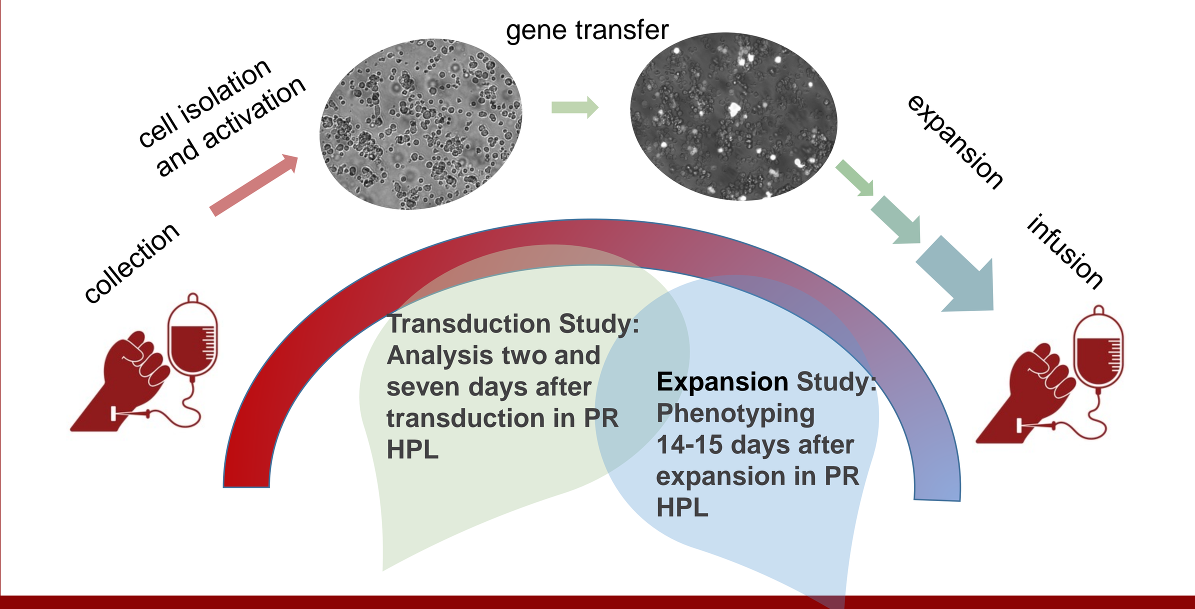
Adoptive immunotherapy with T lymphocytes (T cells) modified *ex vivo* has emerged as a promising therapeutic strategy to treat various cancer and autoimmune diseases. T cells engineered to express chimeric antigen receptors (CARs) have shown high rates of clinical response in patients with hematological 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 leukopheresis, 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 xeno-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) for the *ex vivo* modification and expansion of human primary T cells. PR HPL is produced with a method that has been demonstrated to reduce pathogens, including enveloped and non-enveloped viruses.

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



Key Reagents

- Human Peripheral Blood Pan-T cells, STEMCELL™ Technologies
- Basal medium: CTS™ AIM V™, ThermoFisher Scientific
- Supplements (at concentrations indicated in figures):
  - PR HPL, Cook Regentec® (R&D produced)
  - human AB serum-heat inactivated, Sigma H3667
  - FBS, Gibco™ 16000-044
- IL2 (100 U/mL working concentration), STEMCELL Technologies
- Immunocult™ CD3/CD28 T cell Activator, STEMCELL Technologies
- CMV-GFP lentiviral particles were supplied by the Indiana University Vector Production Facility (Dr. Ken Cornetta)

Methods

- Cells were thawed directly from the manufacturer's cryovial or used after a single round of refreezing and rethawing.
- For both the transduction and expansion studies cells were thawed and activated according to manufacturer's instructions in a 12-well plate with media containing IL2 and supplements as indicated in the figures.
- Following a four-day post-thaw recovery period, 100,000 cells in 100uL complete media containing polybrene (8ng/mL) were transduced with lentivirus in a 96-well plate. Analysis was performed by flow cytometry two or seven days following transduction. [for transduction study only]
- For cell expansion/phenotype study, viable cells were counted with a hemocytometer and medium added to adjust total culture volume back to $1x10^6$ cells/mL as needed.
- For flow cytometry data, immunostaining was performed with the following antibody clones using standard procedures: anti-CD4 (rpa-t4), anti-CD8 (rpa-t8), anti-CD45RO (uchl1), anti-CCR7 (151503), anti-CD127 (HIL-7R-M21), anti-CD62L (sk11).
- Four-color flow cytometry was performed on an Accuri C6 or Accuri C6 Plus (BD). Compensation was set based on fluorescence of antibodies bound to AbC™ compensation beads (ThermoFisher). Fluorescence minus one ("FMO") controls and unstained cells were used to define gates.

Lentiviral transduction of GFP reporter transgenes into T cells in human AB serum versus PR HPL

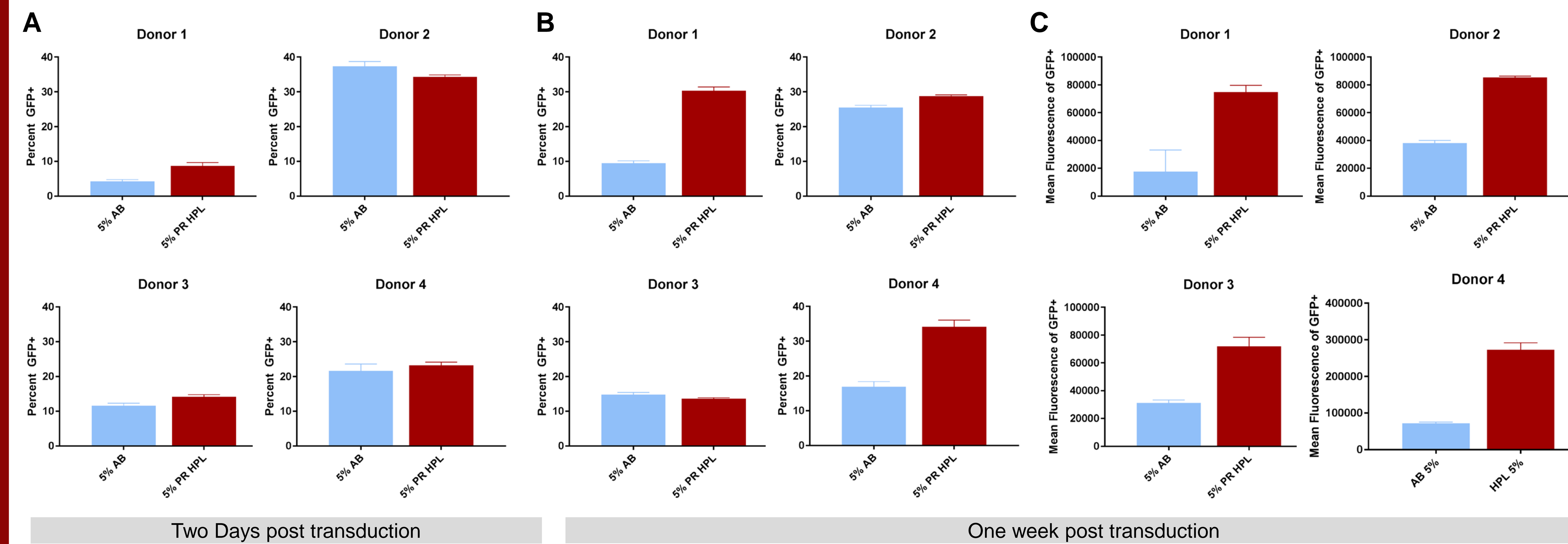


Figure 1. Efficiency of lentiviral transduction of CMV-GFP transgene into T cells in presence of human AB serum or PR HPL. (A) Percent GFP positive two days after transduction, a timepoint when indirect effects of each supplement on culturing would be expected to be minimal. (B) Percent GFP positive one week after transduction, a timepoint when indirect effects of culturing may affect the outcome. (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.

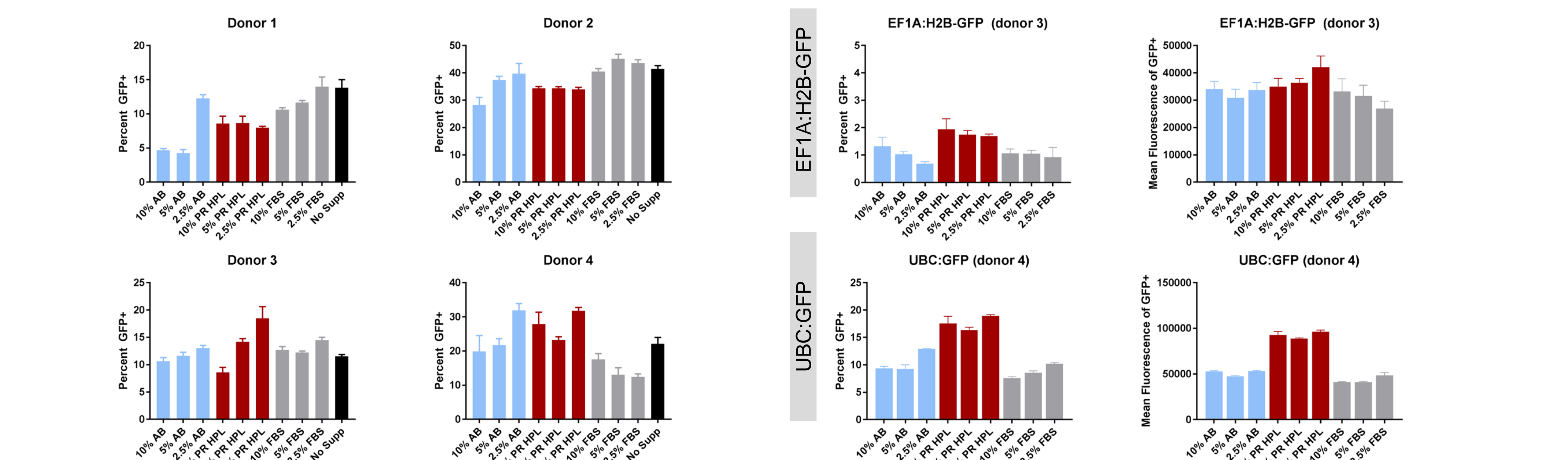


Figure 2. Effect of supplement concentration on CMV-GFP lentiviral transduction. Percent GFP positive two days after transduction. Each graph shows mean and standard deviation for technical replicates (n=3) from one donor. Four donors are shown.

Figure 3. Efficiency of lentiviral transduction of EF1A:H2B-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:H2B-GFP and UBC:GFP) applied to the cells (multiplicity of infection) was not standardized to each other and the data are not meant to be compared across transgenes. Single donor tested per transgene.

Expansion in various supplements

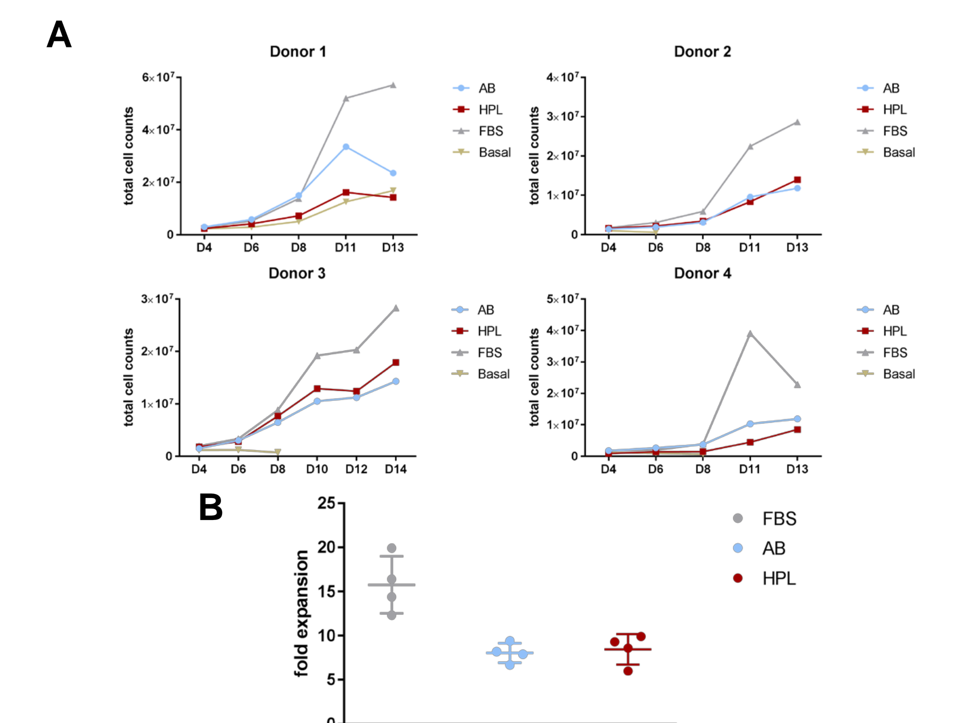


Figure 4. T cell expansion in various cell supplements.
T cells were thawed and activated in media containing 5% of each supplement. Cells were counted and volume measured on the indicated days to obtain total cell counts. (A) Four donors were tested and each plot is shown individually as results varied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

Ratio of CD4 to CD8 cells after culture in various supplements

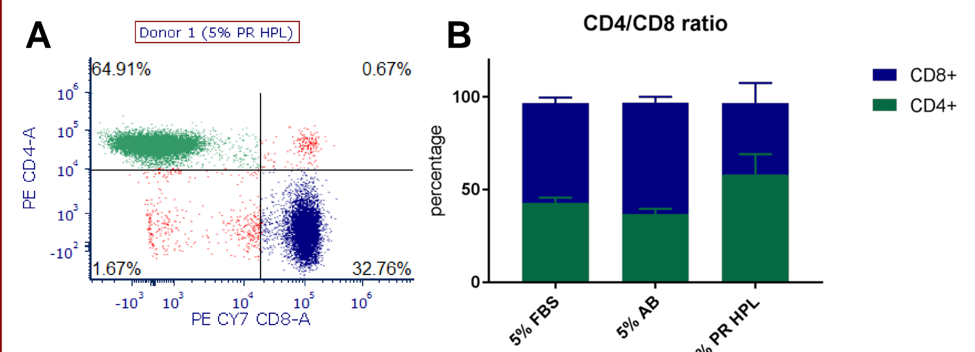


Figure 5. CD4 and CD8 analysis by flow cytometry
(A) Dot plots showing gating on representative data from one donor. Cells shown are from a culture containing 5% PR HPL. (B) Histogram summarizing the mean and standard deviation obtained from four donors analyzed on day 14 after thawing and expansion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

Contact Information

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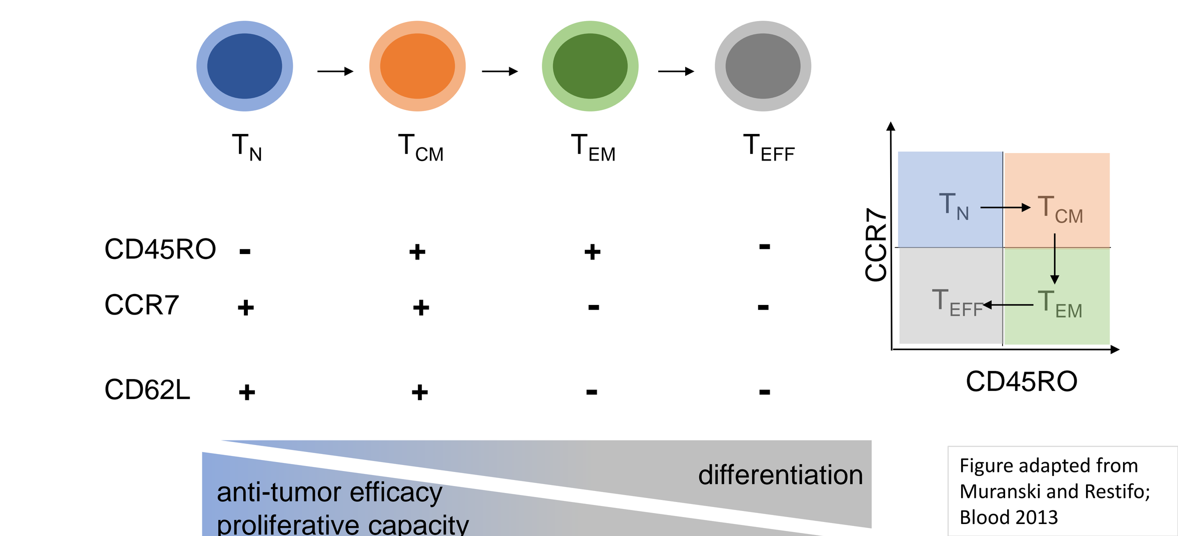
Markers used to assess T cell differentiation



PR HPL supports less differentiated state

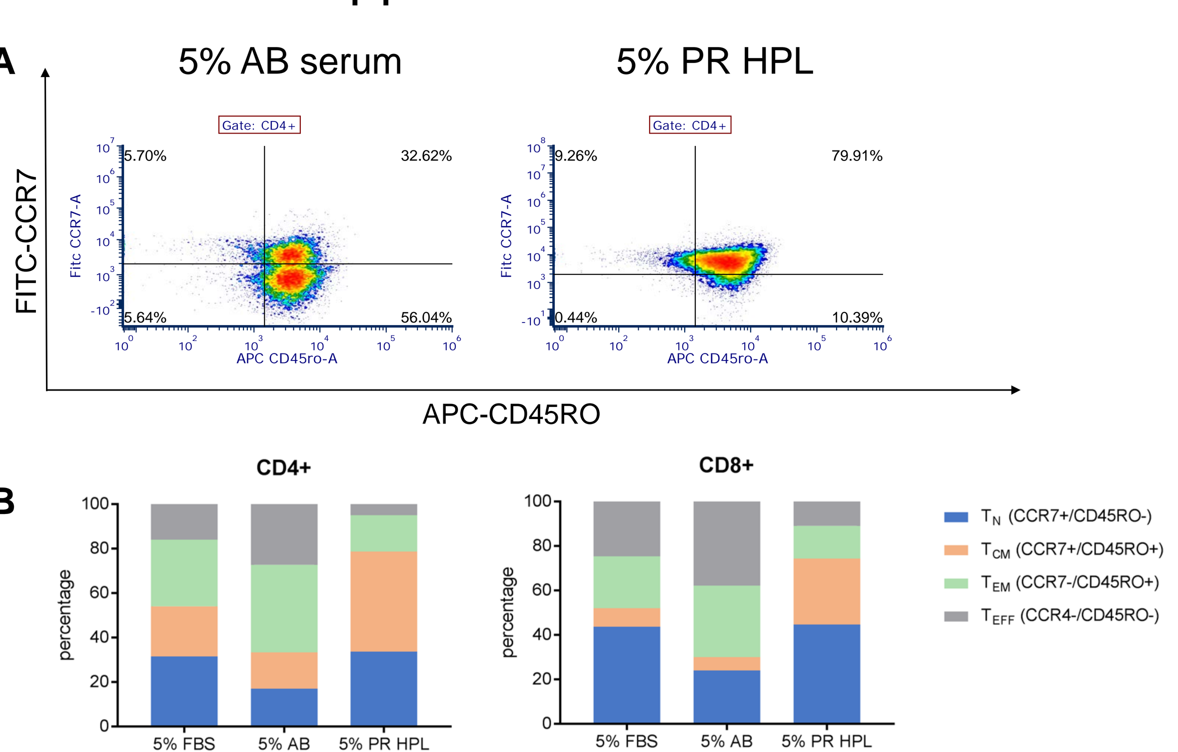


Figure 6. CCR7 and CD45RO analysis by flow cytometry
(A) Representative density/dot plots showing the phenotype of CD4+ gated T cells cultured with PR HPL versus those cultured with AB serum, where the PR HPL has less undesirable differentiation T_{EM} / T_{EFF} (CCR7- cells). All four donors tested showed this shift in phenotype. A similar effect is seen with CD8+ gated T cells (not shown). (B) Summary data showing the proportions of T_N, T_{CM}, T_{EM}, T_{EFF} for CD4+ T cells (left) or CD8+ T cells (right). The means of four experiments are represented in the bar graphs.

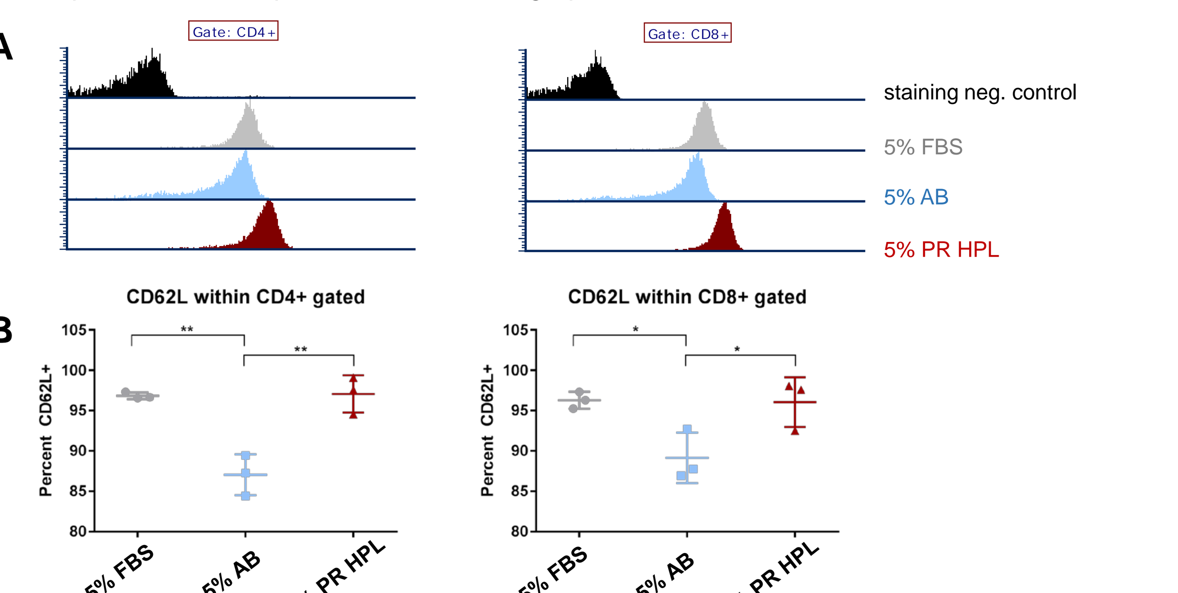


Figure 7. CD62L analysis by flow cytometry
Cells were thawed and cultured in the indicated supplements for 14-15 days prior to analysis. (A) Representative histograms showing distribution of CD62L fluorescence from one donor. Left shows the population gated on CD4+ cells and right is gated on CD8+ cells. (B) Summary data showing mean and standard deviation and individual replicates of percent CD62L+ from three donors. Statistical analyses between groups were carried out using one-way ANOVA. (*=p<0.05, **=p<0.001)

Conclusions

- T 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 40/60-60/40 CD4/CD8 ratio
  - High proportion T_N/ T_{CM} (about 3-5x more T_{CM} than AB serum). T_N/T_{CM} phenotype may be associated with improved long-term tumor killing (Berger *et al.*, J Clin Invest. 2008; Hinrichs *et al.*, Proc Natl Acad Sci USA 2009)
- 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.