Chronic anti-CD3/CD28 Dynabeads stimulation generates exhausted T cells with a distinct metabolic phenotype

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Abstract

T cell exhaustion is a major challenge in cancer immunotherapy, as exhausted T cells lose essential effector function and persistence in the TME. Recent studies showed that impairment of mitochondria function plays a critical role in developing and maintaining T cell exhaustion. These observations prompted us to explore the potential use of metabolic profiling of T cells to assess relative T cell exhaustion.

In our study, we induced exhaustion in previously expanded T cells via in vitro chronic stimulation with anti-CD3/CD28 Dynabeads and used briefly stimulated (24-hour) counterparts as controls. Across all three donors, chronically stimulated T cells exhibited reduced net proliferation, increased percentage of $PD1^{+}Tim3^{+}$ population, and decreased IL-2 secretion upon restimulation, while TNFα and IFNγ secretion varied by donor.

Importantly, when assessed using the Agilent Seahorse XF T Cell Metabolic Fitness assay, these exhausted T cells displayed a distinctive metabolic phenotype, including a significant increase in glycolytic activity, with reduced mitochondrial spare respiratory capacity (SRC). In addition, these cells have a decreased capacity to further increase glycolytic activity upon acute restimulation. These findings suggest that metabolic profiling is a valuable tool for identifying metabolically fit T cells and designing strategies to improve T cell therapies' efficacy, representing a great addition to the current matrix of parameters for assessing T cell exhaustion.

Experimental

XF T Cell Metabolic Profiling Kit

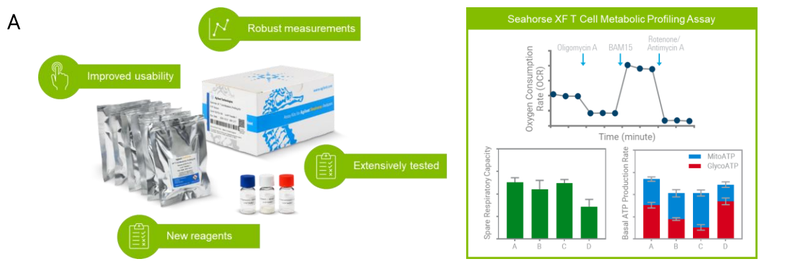

Fig. 1 (A) The Agilent Seahorse XF T Cell Metabolic Profiling kit contains new reagents optimized to allow for complete measurement of T cell metabolism along with a dedicated analysis tool, Agilent Seahorse Analytics. (B-C) BAM15 and optimized concentration of FCCP were compared in mouse naïve CD8 T cells. (B) Changes in extracellular oxygen levels after uncoupler addition, highlighting the more consistent rate during the three minutes of instrument measurement obtained when adding BAM15. (C) OCR kinetic profile, illustrating underestimation of Max respiration obtained when FCCP is used as uncoupler. Similar results were obtained using mouse NK cells, human T and NK cells.

Workflow to generated exhausted T cells in vitro

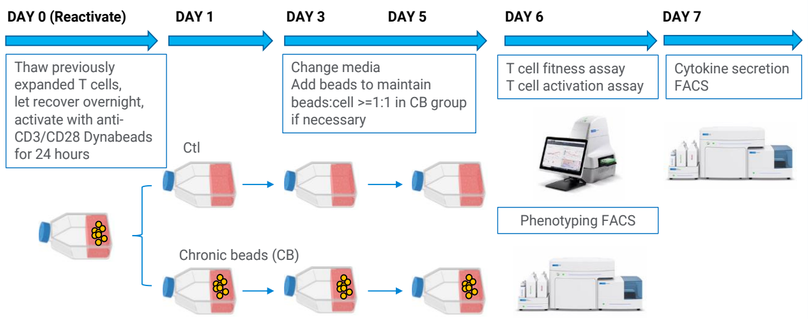

Fig. 2 Workflow to generate exhausted T cells in vitro through chronic beads stimulation Frozen vials of human peripheral blood pan-T cells previously activated and expanded for 14 days were thawed in RPMI-1640 media supplemented with 10% FBS, 0.05 mM beta-mercaptoethanol and 300 U/mL human IL-2. After overnight recovery, T cells were reactivated with anti-CD3/CD28 Dynabeads. 24 hours after reactivation, cells were separated into control group and chronic beads (CB) group. For control group, beads were removed, for CB group, beads were retained during the whole expansion. Samples from three different donors were used for the study. For donor two, cytokine secretion FACS was performed six days instead of seven days after reactivation.

Results

Chronic beads stimulation generates exhausted T cells

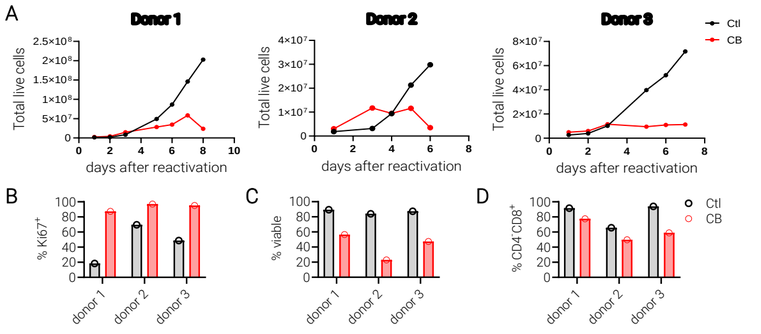

Fig. 3 Chronic beads stimulation significantly impaired net T cell proliferation. (A) Growth curve of control and chronic beads (CB) group from one day to 6-8 days after reactivation. (B) Percent of $Ki67^{+}$, (C) percent viable and (D) percent of $CD4^{-}CD8^{+}$ T cells six days after reactivation.

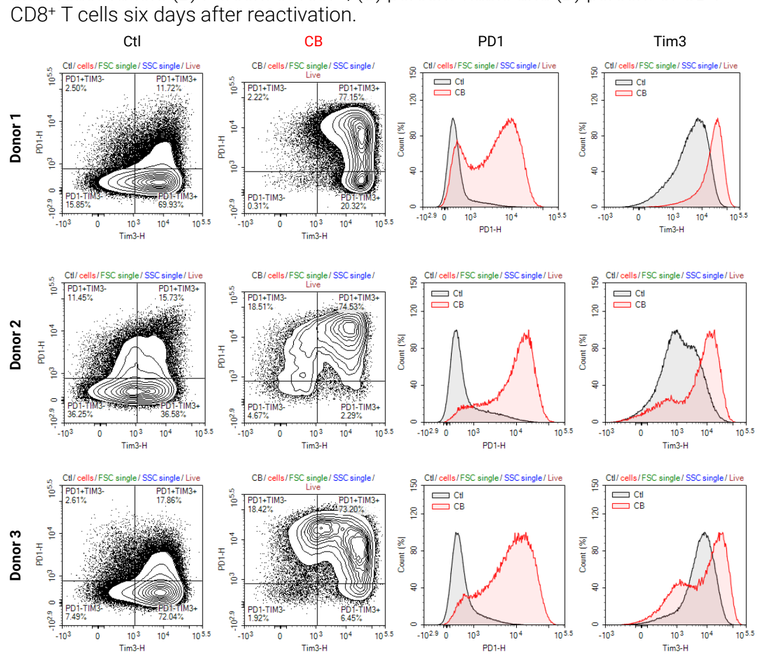

Fig. 4 Chronic beads stimulation significantly increased exhaustion marker expression on T cells . Phenotyping FACS results for T cell exhaustion markers six days after reactivation.

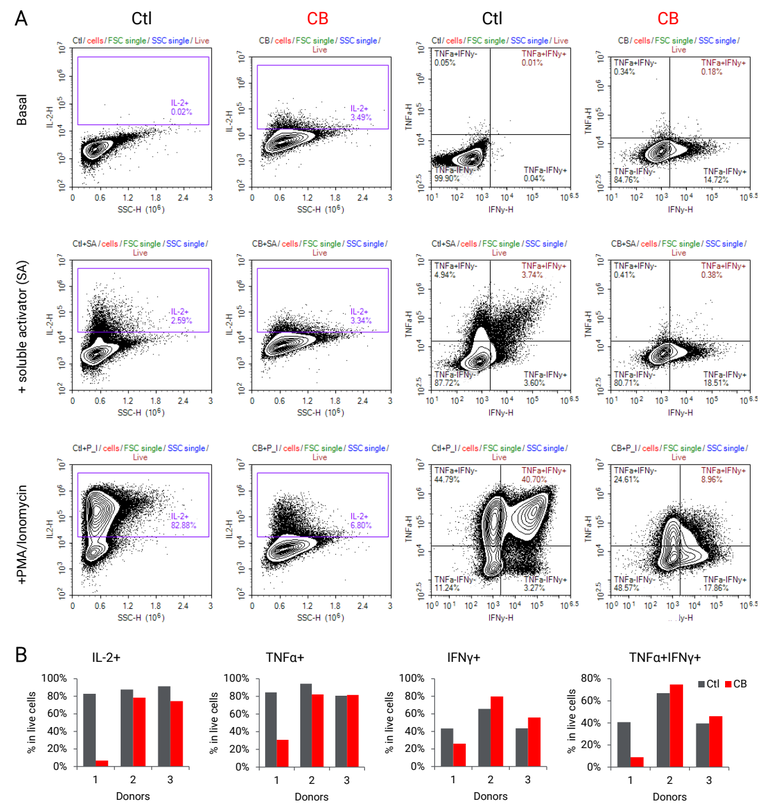

Fig 5. Chronic beads stimulation consistently impaired T cell IL-2 secretion upon restimulation. Six to seven days after reactivation, T cells from control and CB group were treated with vehicle or a soluble activator (SA, ImmunoCult Human CD3/CD28 T cell activator from StemCell), or a combination of phorbol myristate acetate (PMA) and ionomycin for four hours for cytokine secretion analysis. (A) Gating for cytokine secretion seven days after T cell reactivation, representative data in donor one. (B) Percentage of cytokine positive cells after PMA/Ionomycin stimulation in each donor.

Results

Exhausted T cells generated through chronic beads stimulation have a distinct metabolic phenotype

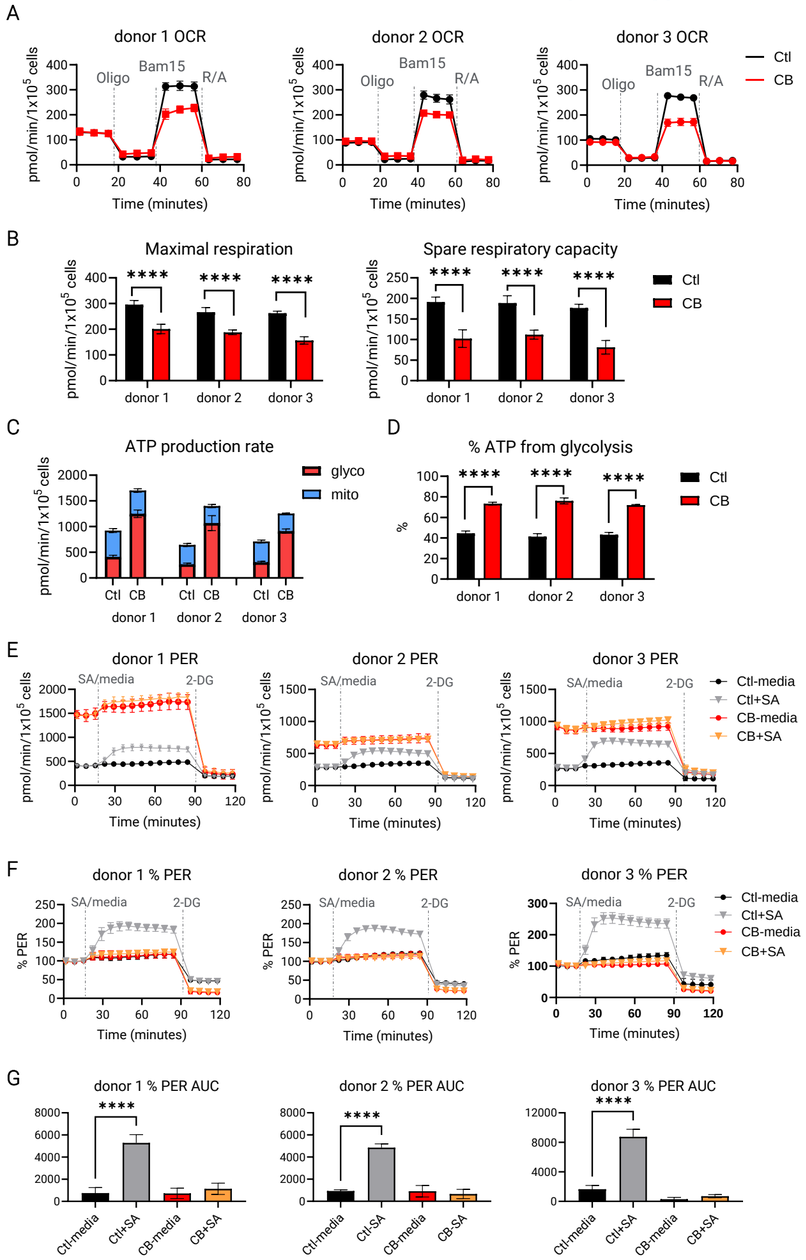

Fig 6. Distinctive metabolic phenotype of exhausted T cells in CB group. (A) OCR kinetic curve and (B) maximal respiration and spare respiratory capacity and (C) ATP production rate and (D) percent ATP from glycolysis in T cell fitness assay six days after reactivation. Oligo: oligomycin-A. R/A: Rotenone/ antimycin. (E-F) Proton efflux rate (PER) in T cell activation assay not normalized (E) or normalized (F) to baseline (measurement 3). (G) Area under the curve (AUC) for percent PER. SA: anti-CD3/CD28 soluble T cell activator. 2-DG: 2-deoxy-glucose. Data are presented as mean ± SD, ****p<0.0001

Conclusions

- Our experimental model of T cells expanded under chronic anti-CD3/CD28 Dynabeads stimulation result in a T cell population with distinctive immunophenotype (increased levels of Tim3 and PD1 expression) and impaired net proliferation.
- Exhausted T cells induced by chronic beads stimulation have a distinct and consistent metabolic phenotype characterized by decreased maximal respiration, decreased spare respiratory capacity and increased glycolytic poise.
- Despite the increased glycolytic activity, exhausted T cells showed reduced capacity to further increase glycolysis activity after acute reactivation, in accordance with cytokine secretion profile observed after restimulation.
- Agilent Seahorse XF assays allow obtaining the complete metabolic phenotype of T cells and identifying cell populations that are metabolically fit, thus providing valuable information to improve cell therapy development.

References

1. Assessing T cell Bioenergetic Poise and Spare Respiratory Capacity Using Extracellular Flux Analysis. https://www.agilent.com/cs/library/applications/an-xf-tcell-metabolic-profiling-kit-5994-4494en-agilent.pdf
2. Using the Agilent NovoCyte Flow Cytometer for Immune Suspension Normalization in Agilent Seahorse XF Assay. https://www.agilent.com/cs/library/applications/an-novocyte-seahorse-5994-6245en-agilent.pdf