LymphomaHub

CLL/SLL

Decreased mitochondrial fitness correlates with impaired CAR-T cell persistence and poorer responses in CLL

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Impaired immune function is a well-known characteristic of patients with CLL causing increased susceptibility to infections and failure of anti-tumor immune responses. However, the reasons for the defective immune response are not fully understood.

On 10 May, Jaco van Bruggen from the University of Amsterdam, Amsterdam, NL, and colleagues, published in *Blood*[1] an exploratory study on the potential effect of impaired T-cell metabolic homeostasis on chimeric antigen receptor T-cell therapy (CAR-T) outcomes in chronic lymphocytic leukemia (CLL).

The authors of the study have previously shown that in CLL, $CD8^+$ T-cells exhibit increased mitochondrial membrane potential and respiration and consequently high levels of reactive oxygen species (ROS). In order to better understand the role of mitochondrial metabolism and biogenesis the authors conducted a study in CD8+ T-cells derived from patients with CLL and healthy volunteers.

Study design

- Peripheral blood marrow cells (PBMCs) were isolated from peripheral blood samples of patients with previously-untreated CLL (N = 62) and age-matched healthy individuals (N = 39)
- CAR-T cells were obtained from N = 27 patients with relapsed or refractory (R/R) CLL who were enrolled in two clinical trials where they received one tisagenlecleucel infusion (Kymriah®; NCT01029366 [n = 21] and NCT01747486 [n = 6])
- Male patients: n = 18
- Patients who achieved complete response (CR) after CAR-T infusion: n = 7
- The main techniques used on the PBMCs and CAR-T cells were flow cytometry, gene expression analysis and glucose/lactate concentration analysis before or after T-cell receptor (TCR) stimulation with anti-CD3/CD28 antibodies

Key findings

$CD8^+$ T-cell activation levels and glucose metabolism

- After *TCR stimulation*, and when compared to control cells, $CD8^+$ T-cells derived from naïve CLL patients showed:
  - Decreased $CD8^+$ T-cell activation markers (CD25, CD38, CD71)
  - Decreased $CD8^+$ T-cell degranulation markers (CD107a)
- An increased frequency of programmed cell death protein 1 $(PD\text{-}1)^+$ cells was observed in samples derived from patients with naïve CLL, before and after TCR stimulation

- Metabolic cellular homeostasis in the form of glucose uptake was impaired following TCR stimulation in $CD8^+$ T-cells derived from naïve CLL patients (decreased GLUT1 surface expression and glucose uptake)
- Similar results were obtained when $CD8^+$ T-cells were stimulated with anti-CD3 antibodies alone, indicating that these results are not affected by potential differences in the levels of $CD28^+$ cells in the samples
- These results indicate that $CD8^+$ T-cells derived from naïve CLL patients show impaired activation and glucose uptake mechanisms

Co-culture experiment

The authors separated the $CD8^+$ T-cells from the CLL cells per sample (average number of CLL cells per sample: 88%) and co-cultured them in two different systems, where the two cell populations were physically separated by a thin membrane or not

- Upon TCR stimulation, the $CD8^+$ T-cells derived from patients with naïve CLL showed impaired glucose uptake only when in contact or in the presence of CLL cells, but not when they were cultured in isolation.
- The reduced glucose uptake observed by the $CD8^+$ T-cells derived from patients with naïve CLL was not due to limited glucose availability or increased lactate levels in the cell culture media
- This result indicated the existence of a potential soluble factor, release from CLL cells that mediates the observed metabolic impairments

Mitochondrial metabolism & biogenesis

- $CD8^+$ T-cells derived from patients with naïve CLL had increased mitochondrial oxidative phosphorylation and increased levels of ATP production, which was not due to changes in mitochondrial size between control and CLL-derived cells
- $CD8^+$ T-cells derived from patients with naïve CLL also had a higher mitochondrial membrane potential and thus increased associated ROS generation, with concomitant decrease in superoxide dismutase 2 (SOD2), a main ROS scavenger
- Upon TCR stimulation, mitochondrial biogenesis was decreased in CLL-derived $CD8^+$ T-cells. This was reversible upon depletion of the CLL cells in the sample
- These results indicate a significant mitochondrial impairment in CLL-derived $CD8^+$ T-cells

Mitochondrial biogenesis in CLL CAR-T cells

- The authors compared $CD8^+$ CAR-T cells from R/R CLL patients who achieved a CR and partial response following infusion, for mitochondrial size and membrane potential, as well as glucose uptake and ROS levels
- Mitochondrial size was the only factor that was significantly larger in patients achieving CR *versus* non-responders
- This increased mitochondrial size correlated with various CAR-T cell persistence markers (CAR-T cell expansion peak, CAR-T fold *in vitro* expansion, area under the curve, peak % of $CD3^+$:$CD8^+$ cells in the first 28 days after infusion)
- In this experiment, mitochondrial size works as a mitochondrial biogenesis indicator since T-cells have been activated during the CAR-T procedure

- These results indicate that mitochondrial mass might be a marker for CAR-T outcomes in patients with CLL

Conclusions

According to the authors, $CD8^+$ T-cell from patients with CLL had increased mitochondrial oxidative phosphorylation and increased levels of ATP production. After stimulation these cells had also reduced glucose uptake and mitochondrial biogenesis indicating impaired function. In patients with CLL receiving CAR-T cells increase of mitochondrial size of $CD8^+$ T-cells correlated with good responses. Therefore, mitochondrial size could be a marker for CAR-T outcomes in patients with CLL. Moreover, the authors hypothesize that enhancing the mitochondrial biogenesis of $CD8^+$ T-cells during CAR-T cell production might lead to a better outcome in patients with CLL

References

1. van Bruggen J.A.C. *et al.* Chronic lymphocytic leukemia cells impair mitochondrial fitness in CD8+ T cells and impede CAR T cell efficacy. *Blood.* 2019 May 10. pii: blood.2018885863. DOI: 10.1182/blood.2018885863 [Epub ahead of print]
2. Siska P.J. *et al.* Suppression of Glut1 and Glucose Metabolism by Decreased Akt/mTORC1 Signaling Drives T Cell Impairment in B Cell Leukemia. J Immunol. 2016 Sep 15;197(6):2532-40. DOI: 10.4049/jimmunol.1502464 [Epub 2016 Aug 10]