Dynamic cardiolipin synthesis and remodeling is required for CD8+ T cell immunity
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Q&A

Q: Did the CD8+ T-cell differences in Barth patients correlate with alterations in neutrophil counts (relative or absolute?) Or Monocyte counts?
A: Unfortunately, we did not have access to these data.

Q: Does only CL increase? How about other lipids such as PE and plasmalogen?
A: Plasmanogen was not included in our targeted lipidomics. We did not observe any overall increase in PE during memory T cell development.

Q: What is the function of spare respiratory capacity in the T cell?
A: Spare respiratory capacity allows memory T cells a rapid recall and reactivation after a second challenge by a known antigen, which is a hallmark of immune memory.

Q: Why do you look at alanine instead of lactate in the PTPMT DT/Wt mouse?
A: We also observed an increase in 13C-glucose derived carbons in lactate in PTPMT1 deficient T cells upon activation, although a lower level compared to the 13C-glucose derived carbons accumulating in alanine. It is important to keep in mind that we measured intracellular metabolites and lactate, when it reaches too high intracellular levels, gets pumped out of the cells as detoxifying mechanism, so we might lose it for our analysis.

Q: How does the fatty acid side chain composition change during activation?
M: In the first 6h of activation, we observe a reduction of CL 72:9, 72:6, 70:6, 70:9. Starting from 24h after activation we observe an overall decrease in all CL forms (72:8, 76:8, 72:7, 74:8). Later at 48h after activation, while overall CL levels remain below the ones from non-activated cells, some CL species levels start to increase again (mainly CL 72:6).
Q: Nice talk, my question is if you can say that if patients with barth syndrome don’t respond to vaccines?
A: This is a really good question. From my data from patients, unfortunately I cannot say that but the experiments suggest a possible T cell functional impairment. It is therefore tempting to speculate that Barth Syndrome patients might respond less efficiently to T-cell mediated vaccines. Anyway, this speculation will need to be tested and analyzed in a larger experiment that I hope can conduct with the support of Barth Syndrome Foundation.

Q: What clinical phenotypes might be expected from the observed CD8+ T cell defects?
A: 1) The T cell defect might explain, in combination with neutrophil defects, the susceptibility to recurrent infection. 2) It is tempting to speculate non-efficient response to T cell mediated vaccines. 3) The T cell defect might also explain the lower frequency of acute organ rejection after heart transplant observed by Dr. Yu Li, University of Pittsburgh, and presented during the present symposium.

Q: Fascinating talk, is there some reason you use ptpmt1 deletion instead of cardiolipin synthase deletion? Thank you.
A: We needed a targeted deletion only in T cells otherwise both PTPMT1 and CRLS1 total body knock out mice are not viable. When we started the project the PTPMT1 conditional knock out mouse was the only one available.

Q: Is the number of mitochondria also changing during T-cell development? And if you take it into account when measuring CL.
A: Mitochondrial mass does not change after activation or upon transient nutrient deprivation. It increases during memory T cell differentiation. We took this observation into account and normalized CL mass to mitochondrial mass.

Q: Is there clinical evidence of T-Cell deficiency in Barth syndrome?
A: I think that our analysis is the first one systematically analyzing T cells in mouse models of cardiolipin deficiency and Barth Syndrome patients.

Q: What happens to the level of other phospholipids?
A: This is currently the focus of other projects in our lab.

Q: Have you tried a different substrate which comes in after PDH such as glutamine?
A: Thanks for the question. We did not perform the glutamine supplementation experiment in our model but this experiment is definitely worth performing.
Q: The spare reserve capacity (FCCP) is difficult to interpret given there was no difference in oligo (an indirect measure of actual ATP synthesis). The data would suggest that ATP synthesis is similar between groups.
A: I imagine this question refers to the higher spare respiratory capacity observed upon transient glucose starvation. It is known that short-time glucose starvation induces higher reliance on mitochondrial respiration. So, although glucose levels are lower, cells increase mitochondrial-derived ATP production to sustain viability (Gomes et al, Nature Cell Biology, 2011). Thus, it is not surprising that although lower glucose availability is reduced, starved cells maintain a comparable ATP-coupled respiration measured after oligo. The higher efficiency in mitochondrial respiration during transient glucose starvation is also reflected by the higher maximal respiration after FCCP uncoupling.

Q: Deficits in memory T-cells in some other mitochondrial diseases causes common variable immune deficiency. Does CVID occur in Barth syndrome?
A: Unfortunately, this question is out of my expertise and I would need to turn it to physicians more expert on patients’ status.