

Assessing the impact of the nutrient microenvironment on the metabolism of effector CD8⁺ T cells

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SCOPE OF THE METHOD

The Method relates to	Human health
The Method is situated in	Basic Research
Type of method	In vitro - Ex vivo
Species from which cells/tissues/organs are derived	Mice
Type of cells/tissues/organs	Spleen

DESCRIPTION

Method keywords

Nutrient
Microenvironment
Metabolism
CD8+ T cells
Physiological blood-like medium
cell culture
Metabolic flux
Metabolic quenching
Custom media formulations

Scientific area keywords

Immunometabolism
CD8+ T cells
13C tracer analysis
Nutrient microenvironment

Method description

The method is an approach to systematically study the impact of the nutrient microenvironment on the metabolism of effector CD8+ T cells, based on performing stable 13C isotope labeling measurements on *in vitro*-differentiated murine effector CD8+ T cells. Naive CD8+ T cells are isolated from mouse spleens, further activated and differentiated into an effector state *in vitro*. Effector CD8+ T cells are then cultured under different medium conditions, in the presence of 13C isotope tracers. Intracellular metabolites are extracted from these cells, and 13C-label incorporation patterns and metabolite levels are determined via MS-based analysis. Coupling this information with growth rates, metabolite uptake and secretion rates, and an appropriate metabolic flux model, you will be able to assess the impact of the different nutrient conditions on the metabolism of effector CD8+ T cells.

Lab equipment

Chemical fume hood ;
Biological safety cabinet ;
pH meter ;
Centrifuge fitting 15 and 50 mL tubes ;
Humidified, temperature and CO2-controlled cell culture incubator ;

Vacuum aspirator ;

Refrigerated centrifugal vacuum concentrator;

Mass Spectrometer with gas or liquid chromatographic technique.

Method status

Published in peer reviewed journal

PROS, CONS & FUTURE POTENTIAL

Advantages

A key feature of the method lies in the cell culture medium formulation, or Blood-Like Medium (BLM). The latter has been adapted from the literature to match the concentrations found in human plasma for a variety of nutrients, in order to provide results more representative of physiological conditions. In addition, the described BLM formulation has the further advantage of being easily customizable, allowing to pull out a variety of individual nutrients (in particular, several non-essential amino acids, glucose, and pyruvate) with minimum work. This enables replacing these nutrients with ¹³C-labeled analogs of choice, or to investigate the impact of the full or partial depletion of any (or a combination) of them on the metabolism of effector CD8⁺ T cells. You reduce the amount of mice used by testing different conditions in culture on T-cells from one mouse.

Challenges

This technique still doesn't fully recapitulate the *in vivo* immune response although it is already a step in the good direction. For this method, you will still need to collect a mouse spleen. T-cells are small cells which means you will need an adequate number of cells to get accurate metabolite measurements. T-cells are cultured in suspension. Metabolism is a rapidly adapting process. Therefore, it is critical to work as fast as possible during the metabolic quenching. Metabolic quenching of cells in suspension takes longer than quenching attached cells. This will increase the chance of perturbations to cellular metabolism.

Future & Other applications

The easily customizable blood-like medium can also provide results more

representative of physiological conditions in other applications.

REFERENCES, ASSOCIATED DOCUMENTS AND OTHER INFORMATION

Links

[Assessing the Impact of the Nutrient Microenvironment on the Metabolism of Effe...](#)

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