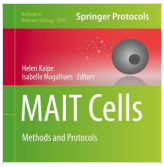

Sep 22, 2021

MAIT Cell Adoptive Transfer

In 1 collection

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ABSTRACT

This is part 3.2 of the "Study of MAIT Cell Activation in Viral Infections In Vivo" collection of protocols.

Collection Abstract: MAIT cells are abundant, highly evolutionarily conserved innate-like lymphocytes expressing a semi-invariant T cell receptor (TCR), which recognizes microbially derived small intermediate molecules from the riboflavin biosynthetic pathway. However, in addition to their TCR-mediated functions they can also be activated in a TCR-independent manner via cytokines including IL-12, -15, -18, and type I interferon. Emerging data suggest that they are expanded and activated by a range of viral infections, and significantly that they can contribute to a protective anti-viral response. Here we describe methods used to investigate these anti-viral functions in vivo in murine models. To overcome the technical challenge that MAIT cells are rare in specific pathogen-free laboratory mice, we describe how pulmonary MAIT cells can be expanded using intranasal bacterial infection or a combination of synthetic MAIT cell antigen and TLR agonists. We also describe protocols for adoptive transfer of MAIT cells, methods for lung homogenization for plaque assays, and surface and intracellular cytokine staining to determine MAIT cell activation.

ATTACHMENTS

Study of MAIT Cell Activation in Viral Infections In Vivo.pdf

DOI

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EXTERNAL LINK

https://link.springer.com/protocol/10.1007/978-1-0716-0207-2_17

PROTOCOL CITATION

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COLLECTIONS

Study of MAIT Cell Activation in Viral Infections In Vivo

KEYWORDS

Virus, MAIT cell, Flow cytometry, MR1-tetramer, Infection, Mouse

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Study of MAIT Cell Activation in Viral Infections In Vivo

MATERIALS TEXT

For materials, please refer to the Guidelines section of the "Study of MAIT Cell Activation in Viral Infections In Vivo" collection.

SAFETY WARNINGS

Personal protective equipment (PPE) should be worn at all times (gloves, lab coat, & eye protection) (*see* **Notes 3** and **4**).

For hazard information and safety warnings, please refer to the SDS (Safety Data Sheet).

BEFORE STARTING

As MAIT cells are to be used for adoptive transfer, all procedures should be performed in a BSCII biosafety cabinet. All tools and reagents should be sterile.

1 7 days or more after intranasal infection with *S.* Typhimurium, MAIT cells can be harvested (*see* **Note 9**).

As MAIT cells are to be used for adoptive transfer, **all procedures should be performed in a BSCII biosafety cabinet. All tools and reagents should be sterile.**

2

Prewarm collagenase media and shaking incubator to **37 °C** .

3 Mice should be euthanized (e.g., using a rising concentration of CO_2 with a second method to confirm death).

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4 Open the diaphragm by cutting the rib cage to expose both the heart and lungs. Gently perfuse the right ventricle with **8 mL** – **10 mL ice-cold RPMI** to dispense circulating blood. Perfuse using a 10-mL syringe and a 26-G needle. Efficient perfusion will result in lung inflation and a color change to pink/white.

5 Remove lungs using scissors to cut through the hilum and place into a 24-well plate containing ice-cold RPMI to transfer organs to the laboratory.

6 Chop lungs into fine pieces (*see* **Note 10**).

7 

Place lung tissue into a 1-mL Eppendorf tube containing **1–2 mL/lung** of pre-warmed collagenase medium. Incubate tubes on their sides in a shaking incubator at **37 °C** , at **100 rpm** – **180 rpm** , for **01:30:00** .

8 During this time prepare Percoll gradients and antibody cocktails (*see* Table 1).

9 After **01:30:00** pour digested tissue through a 70-µm cell strainer and force through into a Petri dish with the plunger from a 1-mL syringe. Rinse residual sample with extra FACS buffer for maximum MAIT cell yield. Cells from multiple lungs (if required) (*see* **Note 11**) are pooled into a single 50-mL Falcon tube with a **total** of **50 mL sterile FACS buffer** .

10 

Centrifuge at **400 x g** for **00:05:00** to pellet the cells. Pour off supernatant (SN).

11 

Resuspend cells in **20 mL 40% Percoll** . Underneath this layer use a transfer pipette to layer **20 mL 70% Percoll** (*see* **Note 12**). Centrifuge this gradient at **800 x g, Room temperature , 00:20:00** with the centrifuge brake OFF. Lymphocytes and other immune cells will form a visible interphase layer between the 40% and 70% Percoll post centrifugation.

12 

During this centrifugation step, prepare single color controls. It is convenient to use part of a spleen forced through a 70-µm filter and resuspended in **5 mL TAC** for **00:05:00** at **37 °C** , then washed once with **5 mL FACS buffer** .

13 

Collect the interphase between 40% and 70% Percoll into a fresh 50 mL Falcon and top up with FACS buffer to a total of

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50 µl . Centrifuge at **400 x g, 00:05:00** .

14

Pour off supernatant and resuspend in **5 mL FACS buffer** , transferring to a 10 mL Falcon tube. Centrifuge at **400 x g, 00:05:00** .

15 Resuspend all lung cells in **750 µl FACS buffer** .

16

Block non-specific tetramer binding by adding **7.5 µl 2.4G2 (anti CD16/32) cell culture supernatant** , containing MR1-6- FP tetramer [8, 16] (no fluorochrome, 1:100). Incubate at **Room temperature** on a roller or bench rocker for **00:15:00** .

17 For lungs from 5 mice, add **750 µl staining cocktail** (Table 1).

18 Cover in aluminum foil to protect fluorochromes from light and shake on roller for **00:30:00** **Room temperature** .

19

Wash with **10 µl FACS wash** . Centrifuge at **400 x g, 00:05:00** . Pour off supernatant.

20

Wash again with **10 mL FACS wash** . Centrifuge at **400 x g, 00:05:00** . Pour off supernatant.

21 Resuspend cells in **2 mL FACS wash** and filter through 40 µm filter into non-pyrogenic FACS tubes.

22

Sort live MAIT cells (defined as $CD3^{+}CD45^{+}$MR1-5-OP-RU tetramer^{+} cells) (Fig. 1) into **3 mL FCS** in 15 mL Falcon tube. For detailed gating strategy, refer to [17]. Wash cells and adjust cell concentration to 5×10^5 cell/mL, allowing 10^5 in **200 µl** for injection to each mouse.

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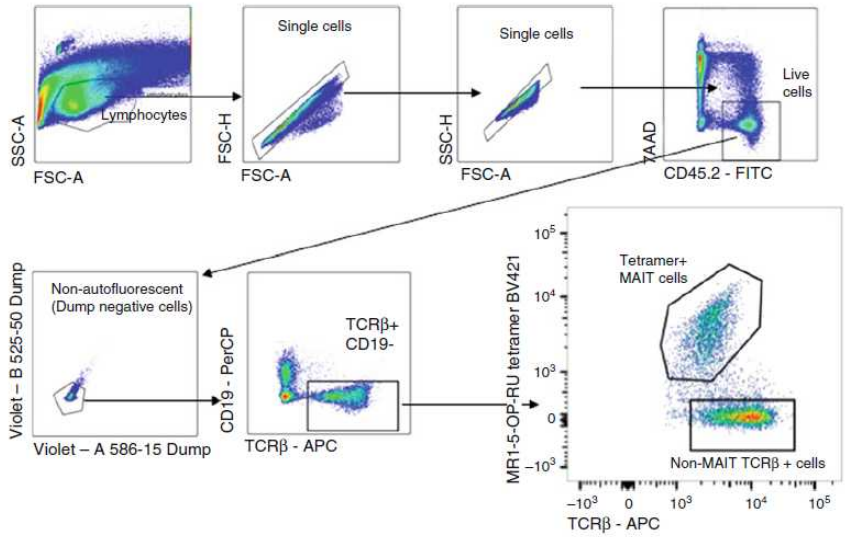

Fig. 1 Flow cytometry gating strategy for MR1-5-OP-RU-tetramer+ MAIT cells

23 Inject 10^5 cells into the tail vein of recipient mice using cells suspended in **200 µl PBS** in a 1-mL syringe with a 26-G cannula after warming the mice for **00:05:00** – **00:15:00** with appropriate monitoring.

24 To deplete residual non-MAIT T cells (*see* **Note 13**), inject recipient mice on days 2 and days 5 or 6 with **0.1 mg** each of purified anti-CD4 (GK1.5) and anti-CD8 (53.762) monoclonal antibodies i.v.

25 Rest mice for a total of 2 weeks post adoptive transfer to allow MAIT cell populations to settle in the host.

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