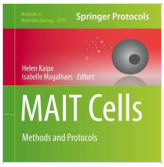

Sep 22, 2021

Influenza A Virus Infection

In 1 collection

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ABSTRACT

This is part 3.3 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

dx.doi.org/10.17504/protocols.io.bmgyk3xw

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).

1 Thaw virus vial rapidly in a **37 °C** water bath until all ice crystals have melted.

2 Decontaminate the outer surface of the vial with **70 % ethanol** .

3

Perform serial dilutions in sterile PBS to achieve the desired inoculum. For example:
(a) If titer of PR8 stock = 1.0×10^9 plaque-forming units (PFU)/mL, require 25 PFU/inoculum (*see* **Note 14**).
(b) Volume of inoculum required for intranasal infection = 50 µL/inoculum.
(c) 25 PFU/50 µL = 500 PFU/mL, dilution required from virus stock DF = 2,000,000.

- Dilution 1: 1/100: **10 µl virus stock** + **990 µl PBS** .
- Dilution 2: 1/100: **10 µl dilution 1** + **990 µl PBS** .

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- Dilution 3: 1/100: **10 µl dilution 2** + **990 µl PBS** .
- Dilution 4: ½: (depends on volume required, e.g., 20 inoculations = 1000 µL) **500 µl dilution 3** and **500 µl PBS** .

4 Mix virus with vortex before administering the inoculum.

5 Infect mice i.n. with **50 µl** under isoflurane anesthesia (*see* **Note 7**).

6 Allow mice to recover and monitor mice for until recovery (typically 10 days) (*see* **Note 15**).

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