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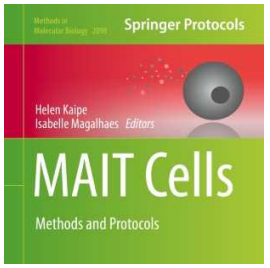
Lung Homogenization



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Timothy S C Hinks[1], Bonnie van Wilgenburg[2], Huimeng Wang[3], Liyen Loh[3], Marios Koutsakos[3], Katherine Kedzierska[3], Alexandra J. Corbett[3], Zhenjun Chen[3]

[1]Respiratory Medicine Unit, Nuffield Department of Medicine Experimental Medicine, University of Oxford, Oxfordshire, UK;
[2]Peter Medawar Building for Pathogen Research and Translational Gastroenterology Unit, Nuffield Department of Clinical Medicine, University of Oxford, Oxford, UK;
[3]Department of Microbiology and Immunology, The Peter Doherty Institute for Infection and Immunity, The University of Melbourne, Parkville, Australia

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Abstract

This is part 3.4 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

PDF

Study of MAIT Cell A...

386KB

Materials

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

Troubleshooting

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 Collect the lungs into 2 mL RPMI supplemented with penicillin/streptomycin .

2 For homogenization, place the lung and the 2 mL media into 10 mL falcon tubes with lids (*see* **Note 16**).

3 Prepare 10 or 15 mL Falcon tubes with 2 × 5 mL 80%w/v EtOH for cleaning the homogenization probe initially and 1 tube containing HBSS. For each group of samples, prepare further 1 × 5 mL EtOH and 1 × 5 mL HBSS, and for the final probe clean set up 2 × 5 mL EtOH .

4 Homogenize the sample using a homogenizer, mounted on a retort stand with the probe set to medium for 00:00:30 per sample. Keep samples On ice (*see* **Note 17**).

5 Centrifuge the samples at 1000 x g, 00:07:00 .

6 Using a 1 mL pipette, carefully draw up approximately 1 mL supernatant (a little bit more is good), avoiding the pellet and fatty residue on top. Divide this volume into two 1.5 mL Eppendorf tubes. Store at -80 °C for subsequent plaque assays.