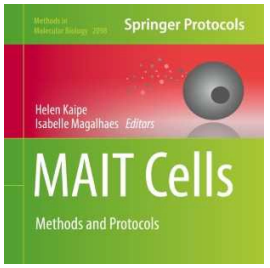
Sep 22, 2021

Study of MAIT Cell Activation in Viral Infections In Vivo



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We use this protocol and it's working

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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

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Study of MAIT Cell A...

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Guidelines

1 Introduction

MAIT cells are relatively recently described innate-like lymphocytes, with similarities to the invariant natural killer T (iNKT) and γδ T cell subsets [1–4]. They are the most abundant innate-like population in the lungs in humans [5] though relatively rare in specific pathogen-free mice [6] and show a striking evolutionary conservation between diverse species of mammals [7]. MAIT cells express a semi-invariant T cell receptor (TCR), which recognizes microbially derived small molecule intermediates from the riboflavin biosynthetic pathway [1, 4, 8, 9]. These molecular intermediates exist only in microbes but not in mammals, and therefore constitute a signature of microbial infection. This property implicates MAIT cells in anti-bacterial host defense, and potentially also in other roles such as tissue repair [3]. However, in addition to their TCR-dependent functions, they can be activated in a TCR-independent manner via cytokines including IL-12, -15, -18, and type I interferon [10–12]. Emerging data suggest that they are expanded and activated by a range of human viral infections including dengue, hepatitis C, and influenza virus [11, 13]. It was not clear from observational human studies whether this would lead to enhanced immune protection, or, conversely, contribute to immunopathology. To address this question, we conducted experimental influenza A virus challenge in vivo in mice and demonstrated that MAIT cells could contribute to a protective anti-viral response [12]. Here we describe the methods used to investigate these antiviral functions in vivo in murine models. To overcome the technical challenge that MAIT cells are rare in specific pathogen-free laboratory mice, we describe (1) how pulmonary MAIT cells can be expanded using intranasal (i.n.) bacterial infection or a combination of synthetic MAIT cell antigen and TLR agonists as well as protocols for (2) adoptive transfer of MAIT cells, (3) viral preparation and infection of mice, (4) lung homogenization, (5) surface and intracellular cytokine staining to determine MAIT cell activation, and (6) plaque assays.

2 Materials

2.1 Reagents and Buffers

1. Antibodies are specified in Tables 1–4.
2. Collagenase medium: Roswell Park Memorial Institute medium (RPMI) containing [M] 3 mg/mL collagenase III, [M] 5 µg/mL DNase, and [M] 2 % fetal calf serum (FCS). Aliquots can be frozen at -20 °C.
3. Fluorescence activated flow cytometry (FACS) buffer: phosphate buffered saline (PBS), [M] 2 millimolar (mM) EDTA, [M] 0.5 % bovine serum albumin (BSA). From a 500 mL bottle of PBS, add 40 mL to a 50 mL falcon containing 2.5 g BSA powder, vortex hard, then filter sterilize back into PBS bottle using a syringe through a 0.22-µm filter. Do not add azide as will be toxic to the cells.
4. Percoll (Density 1.13 g/mL) [M] 40 % and [M] 70 % solutions, pre-warmed to Room temperature for each use.
5. RPMI with pen/strep: RPMI containing [M] 100 µg/mL streptomycin and [M] 100 U/mL penicillin.

6. Tris-based Ammonium Chloride (TAC)–HCl, pH 7.5 hypotonic red blood cell lysis buffer: [M] 0.14 Molarity (M) NH4Cl , [M] 0.017 millimolar (mM) Tris (pH 7.5), then adjust pH to pH 7.2 with HCl ([M] 2 Molarity (M)). The solution is filter (0.22 µm) sterilized and kept at Room temperature .
7. Fixation buffer: [M] 1 % formaldehyde , [M] 2 % glucose in PBS . Fully dissolved solution is kept cold (4 °C) and dark (aluminum foil wrapped) as formaldehyde is sensitive to light.
8. Media for growing MDCK cells: Dulbecco Modified Eagle Medium (DMEM) containing [M] 2 millimolar (mM) L-glutamine , [M] 1 millimolar (mM) MEM sodium pyruvate , [M] 100 U/mL penicillin/streptomycin , and [M] 10 % heat-inactivated FCS .
9. Serum-free (SF) DMEM: Dulbecco Modified Eagle Medium (DMEM) containing [M] 2 millimolar (mM) L-glutamine , [M] 1 millimolar (mM) MEM sodium pyruvate , and [M] 10 U/mL penicillin/streptomycin .
10. [M] 2 X Leibovitz's L-15 media for overlay, make 2x stock as it will be diluted 1:1 with agarose. For 1 L: Use 1 L sterile water . Remove 100 mL of the water but keep for later use. Add two 14 g packets of L-15 powdered media (kept at 40 °C). Add magnetic flea and stir for 04:00:00 or more to ensure the powder is completely dissolved. Adjust pH to pH 6.8 using [M] 1 Molarity (M) HCl . Then add the following to the medium. *(a)* 8 mL 7% w/v NaHCO3 prepared in Hanks Buffered Saline Solution (HBSS) (stored at 4 °C). *(b)* 800 µL 1 M HEPES buffer (pH 6.8) . *(c)* 20 mL 10,000 U/mL Pen/Strep . *(d)* Make up the volume to 1 L (using the 100 mL previously removed) and filter sterilize. Store at 4 °C . To reduce precipitation, aliquot into 50 mL tubes for storage.
11. [M] 1 mg/mL trypsin : warm up trypsin powder for 00:30:00 at Room temperature (kept at 4 °C). Weigh out 10 mg powder and dissolve in 10 mL PBS . Filter using 0.45 µm filter. Aliquot aseptically into 220 µL /aliquot. Store at 20 °C . 200 µL will be added to 100 mL overlay (50 mL L-15 and 50 mL agarose) for a final concentration of [M] 2 µg/mL trypsin/well .
12. Salmonella: *Salmonella enterica*, serovar Typhimurium (attenuated strain BRD509) [14], stored at -20 °C in Luria-Bertani (LB) broth with [M] 50 % glycerol , to prevent freezing at this temperature.
13. MR1-tetramers (5-OP-RU and 6-FP) are available from the NIH core tetramer facility, on application. Store in component parts at -80 °C until ready for use, at which point small aliquots can be tetramerized and stored at 4 °C for days to weeks. They should be reconstituted according to instructions supplied with the product. Typically a 5 µg aliquot of MR1-5-OP-RU monomer or MR1-6-FP monomer should be expanded to a total volume of 18 µL in Tris-buffered saline. About 6.8 µL of commercially available streptavidin-PE at [M] 0.5 mg/mL should be made up to a total volume of 17 µL in Tris-buffered saline. Add 1/10 of the streptavidin-PE solution (1.7 µL) to the monomer solution *every* 00:10:00 and pipette to mix,

incubating at Room temperature in the dark between steps. Repeat until all the streptavidin-PE solution has been added. This will give a final volume of 35 µL containing [M] 0.143 µg/µl tetramer. The tetramer should be titrated for use; typically 1:200–1:1000 dilutions are sufficient.

14. Madin-Darby Canine Kidney (MDCK) cells.
15. Live/Dead Fixable Aqua Dead Cell Stain Kit or Zombie Yellow Viability Stain Kit.
16. Brefeldin A.
17. Phorbol 12-myristate 13-acetate (PMA).
18. Ionomycin.
19. Trypsin–versene.
20. [M] 1 % Crystal Violet in [M] 20 % ethanol and dH_2O.
21. Flow cytometry compensation beads.
22. Flow cytometry 6 µm blank size calibration beads.
23. Fixation/permeabilization buffer and perm-wash buffer.
24. LB agar plates, containing [M] 50 µg/ml streptomycin.
25. LB culture medium.
26. 2.4G2 (anti CD16/32) hybridoma cell culture supernatant.
27. Anti-CD4 (GK1.5) and anti-CD8 (53.762) monoclonal antibodies for depletion of adoptively transferred T cell subsets.
28. [M] 1 % Virkon or [M] 10 % Lysol or Hypochlorite (5000 ppm).
29. [M] 80 % (w/v) EtOH.
30. Hanks buffered saline solution (HBSS).
31. Isoflurane.

Table 1
Flow cytometry panel compatible with a three-laser BD Aria III flow cytometer, allowing identification and sorting of MR1-5-OP-RU-tetramer+ MAIT cells

	Marker	Fluorophore	Laser	Standard dilution if staining in 1500 µL, amount in µL
	CD45.2	FITC	Blue	3.75 µL 1:400
	7AAD	7AAD	Blue or Yellow/Green	3.75 µL *titrate

	CD19	PerCpCy5.5	Blue or Yellow/Green	7.5 µL 1:200
	TCRβ	APC	Red	7.5 µL 1:200
	MR1-5-OP-RU tetramer	BV421	Violet	7.5 µL 1:200

Make up volume to final 720 µL with FACS buffer

Table 2

Flow cytometry panel compatible with a three-laser BD Aria III flow cytometer, allowing optimal identification of MR1-5-OP-RU-tetramer+ MAIT cells using surface stains only

	Marker	Fluorophore	Laser	Standard dilution if staining in 40 µL, amount in µL
	CD45.2 (see Note 1)	FITC	Blue	1:200, 0.2
	TCRβ	APC	Red	1:200, 0.2
	CD19	PerCpCy5.5	Blue or Yellow/Green	1:200, 0.2
	CD8	PE	Blue or Yellow/Green	1:800, 0.08
	CD4	APC Cy7	Red	1:200, 0.2
	MR1-5-OP-RU-tetramer	BV421	Violet	1:200, 0.2

Antibodies should be titrated by each laboratory

Table 3

Surface markers for flow cytometry panel compatible with a three-laser BD Aria III flow cytometer, allowing measurement of MR1-5-OP-RU-tetramer+ MAIT cell activation by intracellular cytokine staining

	Marker	Stain	Laser	Standard dilution if staining in 40 µL,

				amount in µL
	TCRβ	APC	Red	1:200, 0.25
	CD19	PerCpCy5.5	Blue or Yellow/Green	1:200, 0.25
	MR1-5-OP-RU-tetramer	BV421	Violet	1:200, 0.25

Table 4

Intracellular markers for flow cytometry panel for intracellular staining

	Marker	Intracellular stain (see Note 2)	Laser	Standard dilution if staining in 50 µL, amount in µL
	IFNγ	PE Cy7	Blue or Yellow/Green	1:400, 0.125
	TNF	PE	Blue or Yellow/Green	1:300, 0.17
	IL-17	PE or PECy7 or APC (depending on surface stains used)	Blue or Yellow/Green, Red	1:200, 0.25

2.2 Plastic and Other Supplies

1. 1 and 10 mL syringes.
2. 26 G needles.
3. Dissection scissors.
4. 1 mL Eppendorf tubes.
5. 40 and 70 µm cell strainers.
6. 10 cm Petri dishes.
7. 10, 15, and 50 mL Falcon tubes.
8. 5 mL polypropylene or polycarbonate FACS tubes.
9. Flat-bottom 6-well (TC6) plates.
10. 96-well flat-bottom plates.
11. 96-well U- or V-bottom plates.

2.3 Equipment

1. Flow cytometer with capability for cell sorting, BD LSR Aria or equivalent.
2. Spectrophotometer capable of reading at 600 nm.
3. Hemocytometer and light microscope.
4. Animal anesthetic circuit capable of administering volatile inhalational anesthetics.
5. Shaking incubator.
6. Gaseous carbon dioxide and gas exposure chamber.
7. Benchtop mechanical roller for tubes.
8. Tissue homogenizer for disrupting tissue into single cell suspensions.

4 Notes

1. Allow a little extra for pipetting wastage when making up antibody cocktails. Keep on ice and protect from light (e.g., with aluminum foil). Make up cocktails in FACS buffer, but for the intracellular stains these should be made up in Perm Wash buffer containing [M] 0.1 % Saponin .
2. Congenic markers could be reversed or other markers are used as appropriate to the mouse strains being used and to the specific experimental set-up.
3. Biological Hazards—*S.* Typhimurium BRD509 is a risk group 2 pathogen. Influenza A virus-PR8-strain (H1N1) is a lab adapted strain of IAV virus. Work should be risk assessed and we recommend controls that include but are not restricted to the following: Lab coat, safety glasses, and gloves should be worn when performing this protocol. Gloves should be removed or sterilized before exiting the biohazard hood. Solutions of Lysol ([M] 200 Parts per Million (PPM)) or hypochlorite ([M] 5000 Parts per Million (PPM)) should be accessible in case of a spill.
4. Decontaminate all pipette tips in [M] 1 % Virkon when working in the biohazard cabinet. After use, the biohazard hood should be decontaminated by wiping down with [M] 70 % ethanol and by UV sterilization for 00:15:00 before any further use. All waste and its container must be disposed as hazardous waste.
5. Pulmonary MAIT cells can be expanded using any source of 5-OP-RU and an appropriate TLR agonist [15, 17]. A systematic assessment of effective TLR agonists has shown strong MAIT cell expansion 7 days after intranasal inoculation with 76 pmol 5-OP-RU on days 1, 2, and 4 in combination with a single dose of agonist on day 1 to TLR3 (high molecular weight poly I:C), TLR4 (lipopolysaccharide from *E. coli*), TLR2/6 (FSL-1 (Pam2CGDPKHPKSF)), or TLR9 (CpG ODN1826), but not with agonists of TLR1/2 (Pam3CSK4), TLR2, TLR5, TLR7 [3]. Each inoculum should be instilled in 50 µL PBS . However, the requirement for accurate repeated inoculations can introduce significant variability in MAIT cell expansion. A simple, less costly on reagents and time, and equally effective, if not more so, is a single intranasal inoculation with *S.* Typhimurium BRD509 in 50 µL PBS .
6. Growth of bacteria is estimated by measuring the culture in a spectrophotometer at 600 nm. To do so fill a cuvette with fresh LB media, place in spectrophotometer, and use this to blank. Then take 500 µL of bacteria-containing broth and measure optical density. To calculate the inoculum dose, use the estimate that an $O.D._{600nm}$ of 1 = 5×10^8 CFU/mL.

7. Accurate intranasal inoculation depends critically on the depth of anesthesia. Administer isoflurane and observe breathing pattern until respiratory rate has decreased to approximately 100 breaths/min and is deep and relaxed. If insufficient depth is achieved mice will sneeze. If depth of anesthesia is too great (further slowing of respiratory rate and very deep breaths), then mice tend to spontaneously breath-hold and again, volume inhaled will be unreliable. Place 50 µL of inoculum onto the left nasal opening (if user is right-handed) using a P200 pipette, gradually ejecting the 50 µL over a few breath cycles until all has been inspired.
8. Intranasal *S.* Typhimurium is well tolerated in immunocompetent strains such as C57BL/6 and BALB/c with less than 5% of animals showing minor signs of illness (ruffled hair) within 1–2 days after infection. These animals fully recover after days 3–5. The lethal dose of S. Typhimurium BRD509 is >2 × 10^7 CFU/mouse (wild-type C57BL/6 adult). Caution should be used in immunocompromised strains in which pilot experiments should be performed to confirm optimal safe inocula.
9. This MAITcell expansion is long-lived [15], so donor mice can be prepared several weeks in advance.
10. The lungs can conveniently be chopped up using the back of an upturned Petri dish. Using fine forceps lift lungs from the RPMI in which they have been transferred, gently blot off excess liquid with tissue paper and place on the Petri dish. Use a large curved scalpel blade to repeatedly chop through the lungs at multiple angles for at least 00:01:00 each until a very fine and homogeneous texture is achieved.
11. Typically this method will yield 1.5 × 10^6 pulmonary MAIT cells per mouse, so multiple mice may be required as donors, depending on the requirements of the experiment.
12. This will be sufficient for lungs from 8 mice.
13. If transferring cells into a $Rag2^{-/-}\gamma C^{-/-}$ mouse then low frequencies of "contaminating" conventional $CD4^+$ or $CD8^+$ T cells tend to expand more rapidly than the MAIT cells and produce artifacts (not obvious for other T-cell-deficient mice, e.g., $TCR\alpha^{-/-}$ or $RAG2^{-/-}$). As many MAITcells are doublenegative, it is possible to prevent this effect by repeated injections with T-cell-depleting anti-CD4 and anti-CD8 antibodies [17].
14. The PR8 strain of influenza virus is highly virulent in mice and only low inoculate are tolerated. The exact inoculum required for each experimental system will need to be carefully determined depending on the exact strain and batch of PR8 and the strain of mice, and local welfare and monitoring requirements. In our hands C57BL/6 mice receiving 100 PFU of A/PR/8/34 AF18 WCN experienced severe pneumonia in mice, characterized by parenchymal necrosis and infiltrates of macrophages, lymphocytes, and neutrophils, with 10–25% mortality due to welfare concerns or weight loss >20%.
15. Virally infected mice experience a transient viral illness with transient. Viral titers peak at day 3.Weight loss peaks at day 5–7 post infection, and there would be a significant weight gain expected by day 8 and resolving by day 10 post infection. Typically mice should be monitored and/or weighed daily for signs of ill health such as ruffled fur, hunched-up appearance, gait abnormalities, lethargy and loss of body condition for 10 days after challenge or till all the symptoms disappear and body weight returns to pre-challenge level. Monitoring can then return to twice weekly.
16. For many homogenization probes a wide tube is needed, such as the sterile, capped, round-bottom polypropylene tubes which are available.
17. The homogenizer generates a lot of heat at the probe tip. Samples should be kept on ice before and after homogenization, and the probe should be intermittently rested to cool down in ice-cold EtOH between groups of 5 or 10 samples. Between samples or groups of samples clean the probe by running briefly in EtOH and then

rinsing briefly in HBSS. Often connective tissue will clog the probe and this can be removed with large forceps. After use the probe tip should be sterilized.

18. Only approximately 2/7 of one lung is needed for intracellular cytokine staining, so the other lung, or other sections of lung, can be saved for viral titer estimation, histology, or other assays if required.
19. To clarify terminology there are two lungs in each animal, so “one lung” refers to all the 2 or 3 lobes in a single hemithorax. Due to the presence of the heart on the left side, the left lung is smaller with only 2 lobes.
20. Using a spectrophotometer saves time for large numbers of samples. To do this resuspend cell pellet in 1 mL – 2 mL PBS (or adjust according to pellet size/counts). Select $O.D._{600nm}$. Blank cuvette with 1 mL FACS wash/PBS. Measure $O.D._{600nm}$ with 200 µL samples + 800 µL PBS (5×). Calculate the number of cells: this is a simple linear relationship between O.D. and the number of cells, which can be derived by measuring a few cell counts in parallel on both the hemocytometer and the spectrophotometer.
21. An alternative is to resuspend the entire pellet in 700 µL of FACS buffer and take 200 µL into 96-well plate: this should contain approximately 1–1.5 × 10^6 cells, appropriate for staining.
22. To avoid using multiple filters, it is possible to buy large sheets of 40 µm mesh. A single rectangle can be cut which covers a whole plate. Using this, multiple cells can be pipette simultaneously with a multichannel pipette.
23. In round-bottom plates cells may clump so consider using flatbottom plate for the stimulation step, especially if doing further steps in FACS tubes rather than staining in plate format.
24. While surface markers can be measured on the intracellularly stained cells, the most accurate measurement of MAIT cell frequencies will be obtained from immediate surface staining prior to stimulation, due to activation-induced downregulation of the TCR.
25. If cells are not to be acquired immediately, then they can instead be resuspended in 100 µL of fixation buffer and stored at 4 °C until required.
26. This may differ depending on virus and mouse strains.
27. The overlay media will start setting so proceed to the following steps quickly. Overlay media can be made in batches to assist with that.

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References

1. Eckle SB, Corbett AJ, Keller AN et al (2015) Recognition of vitamin B precursors and byproducts by mucosal associated invariant T cells. J Biol Chem 290:30204–30211

2. Godfrey DI, Uldrich AP, Mccluskey J et al (2015) The burgeoning family of unconventional T cells. Nat Immunol 16:1114–1123
3. Hinks TSC, Marchi, E, Jabeen, M et al (2019) Activation and in vivo evolution of the MAIT cell transcriptome in mice and humans reveals tissue repair functionality. Cell Reports 28 (12):3249–3262.e5
4. Kjer-Nielsen L, Patel O, Corbett AJ et al (2012) MR1 presents microbial vitamin B metabolites to MAIT cells. Nature 491:717–723
5. Hinks TS, Zhou X, Staples KJ et al (2015) Innate and adaptive T cells in asthmatic patients: relationship to severity and disease mechanisms. J Allergy Clin Immunol 136:323–333
6. Rahimpour A, Koay HF, Enders A et al (2015) Identification of phenotypically and functionally heterogeneous mouse mucosal-associated invariant T cells using MR1 tetramers. J Exp Med 212:1095–1108
7. Tsukamoto K, Deakin JE, Graves JA et al (2013) Exceptionally high conservation of the MHC class I-related gene, MR1, among mammals. Immunogenetics 65:115–124
8. Corbett AJ, Eckle SB, Birkinshaw RW et al (2014) T-cell activation by transitory neo-antigens derived from distinct microbial pathways. Nature 509:361–365
9. Patel O, Kjer-Nielsen L, Le Nours J et al (2013) Recognition of vitamin B metabolites by mucosal-associated invariant T cells. Nat Commun 4:2142
10. Ussher JE, Bilton M, Attwod E et al (2014) CD161++ CD8+ T cells, including the MAIT cell subset, are specifically activated by IL-12+IL-18 in a TCR-independent manner. Eur J Immunol 44:195–203
11. Van Wilgenburg B, Scherwitzl I, Hutchinson EC et al (2016) MAITcells are activated during human viral infections. Nat Commun 7:11653
12. Wilgenburg BV, Loh L, Chen Z et al (2018) MAIT cells contribute to protection against lethal influenza infection in vivo. Nat Commun 9:4706
13. Loh L, Wang Z, Sant S et al (2016) Human mucosal-associated invariant T cells contribute to antiviral influenza immunity via IL-18-dependent activation. Proc Natl Acad Sci U S A 113:10133–10138
14. Hoiseth SK, Stocker BA (1981) Aromaticdependent Salmonella typhimurium are non-virulent and effective as live vaccines. Nature 291:238–239
15. Chen Z, Wang H, D'souza C et al (2017) Mucosal-associated invariant T-cell activation and accumulation after in vivo infection depends on microbial riboflavin synthesis and co-stimulatory signals. Mucosal Immunol 10:58–68
16. Reantragoon R, Corbett AJ, Sakala IG et al (2013) Antigen-loaded MR1 tetramers define T cell receptor heterogeneity in mucosalassociated invariant T cells. J Exp Med 210:2305–2320
17. Wang H, D'souza C, Lim XY et al (2018) MAIT cells protect against pulmonary Legionella longbeachae infection. Nat Commun 9:3350

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).

Attachments

Study of MAIT Cell A...

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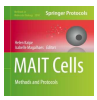

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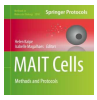

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