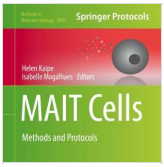

Sep 22, 2021

Viral Plaque Assay

In 1 collection

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ABSTRACT

This is part 3.6 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.

Abstract: Viral plaque assays are used to determine influenza viral titers. A diluted solution of egg-adapted Influenza A viruses/lung-infected tissue homogenates are applied to a six-well tissue culture dish containing a monolayer of Madin-Darby canine kidney (MDCK) cells. The infected MDCK cells grow under a semisolid overlay medium (agar) containing trypsin. A plaque is produced when a virus particle infects a cell, replicates, and then kills the cell. This process can be repeated several times as surrounding cells can be infected by newly replicated virus and killed. When visualized by eye, plaques appear as white spots. The assay is measured in PFU/mL.

ATTACHMENTS

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

DOI

dx.doi.org/10.17504/protocols.io.bmg3k3yn

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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PARENT PROTOCOLS

Part of collection
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).

Passaging MDCK cells:

1 Warm up MDCK cell media, trypsin–versene, and PBS at **37 °C** .

2

Check the confluency of MDCK cells, aspirate the medium, add **10 mL PBS** , aspirate the medium, and repeat wash.

3

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Discard PBS, add 2–3 mL of trypsin–versene (stored **-20 °C**) to MDCK monolayers, and incubate at **37 °C** for **00:05:00** . After **00:05:00** tap the flasks, and incubate for longer if required (maximum **00:15:00**).

4 In the meantime, add **15 mL MDCK cell media** to fresh T75 flasks.

5 Add MDCK cell media to a total volume of **10 mL** to the trypsinized cells, and transfer cells to a 10-mL tube.

6 

Count cells using a hemocytometer.

7 Set up multiple T75 flasks with different cell densities to determine the growth pattern of MDCK cells. Generally ~3–5 × 10^5 for 3-day split.

8 

Incubate at **37 °C** , **5 % CO2** .

Amplification of MDCK cells for plaque assay:

9 Warm up MDCK cell media, trypsin–versene, and PBS at **37 °C** .

10 

Check the confluency of MDCK cells, aspirate the medium, add **10 mL PBS** , aspirate the medium, and repeat wash.

11 

Discard PBS, add **2 mL** – **3 mL trypsin–versene** (stored **-20 °C**) to MDCK monolayers, and incubate at **37 °C** for **00:05:00** . After **00:05:00** tap the flasks, and incubate for longer if required (maximum **00:15:00**).

12 In the meantime, add **40 mL MDCK cell media** to fresh T175 flasks. Set up one T175 flask of MDCK cells per ~4 plates for plaque assay. Each 6-well plate assays 3 viral dilutions (as dilutions are done in duplicate).

13 Add MDCK cell media to a total volume of **10 mL** to trypsinized cells, and transfer cells to a 10-mL tube.

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14 

Count cells using a hemocytometer.

15 Add ~7–8 × 10^5 cells per T175 flask for 3-day culture.

Seeding flat-bottomed 6-well tissue culture (TC6) plates for plaque assay:

16 Warm up MDCK cell media, trypsin–versene, and PBS at **37 °C** .

17 

Check the confluency of MDCK cells, aspirate the medium, add **10 mL PBS** , aspirate the medium, and repeat the wash.

18 

Discard PBS, add **5 mL** – **8 mL trypsin–versene** (**-20 °C**) to MDCK monolayers in T175 flask, incubate at **37 °C** for **00:05:00** . After **00:05:00** , tap bottles, incubate for longer if required (maximum **00:15:00**).

19 Add **17 mL** – **20 mL MDCK cell media** to each flask (total **25 mL** including **5 mL** – **8 mL trypsin**) to inhibit trypsin–versene.

20 Pool cells into one flask.

21 Count the cells, adjust the concentration to **3.3 × 10^5 cells/mL**.

22 Add **3 mL** of 3.3 × 10^5 cells/mL to each well of TC6 plates (~1 × 10^6/well), swirl plates gently to distribute cells evenly. Include a negative control plate.

23 

Incubate cells at **37 °C** , **5 % CO2** **Overnight** . Aim for monolayers to be confluent in 6-well plates for assay.

Plaque assay

24 Warm up SF-DMEM at **37 °C** .

25 

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Prepare dilutions of samples to be titrated. This can be done in a 96-well flat-bottom plate.

26 

Cells will be infected with **150 µl** of each dilution in duplicate, so a minimum of **300 µl** of each dilution is required. A 96-well flat-bottom plate can hold **~350 µL/well**. **35 µl of samples** would be added to **315 µl media** for a final volume of **350 µl** (35 in 350 = 1:10 dilution). If a sample is to be plaqued neat, add **350 µl of sample** to the first well.

27 

Add **315 µl SF-DMEM** in each well of 96-well plate. A multichannel can be used and add **157 µl** and **158 µl** (total **315 µl**) to each well.

28 

Add **35 µl sample** to the first well with media (tenfold dilution) and continue serial tenfold dilutions by transferring **35 µl** across wells, changing tips between dilutions.

29 For titration of viral stocks use dilutions from 10^{-4} to 10^{-6}. 10^{-1} can be used as a positive control. Half-log dilutions can also be performed. For titration of mouse lung homogenates, generally:
(a) Days 1–5: 10^{-1} to 10^{-3}.
(b) Days 6–10: neat to 10^{-2} (*see* **Note 26**).

30 

Wash MDCK cells with **1 mL** – **2 mL** of PBS/well.

31 

Infect cells with **150 µL/well** of the appropriate dilution, swirl gently to cover all cells and incubate at **37 °C** , **5 % CO2** for **01:00:00** , shake gently *every 15 min*.

32 In the meantime, prepare overlay media, best to start doing so in the beginning of the 1 h incubation of MDCK cells.

33 Weigh out **1.8 g agarose** into 200 mL glass bottle, add **100 mL sterile water** (**1.8 % agarose**), and melt in microwave. Store in a **55 °C** water bath.

34 Aliquot **50 mL 2 × L-15 medium** into 50 mL tubes (need 2 × 50 mL aliquots of 2 × L-15 medium and 1 bottle (**100 mL**) of **1.8 % agarose**) and store in **37 °C** water bath.

35 Thaw trypsin–versene at **40 °C** . Thaw a **200 µl** aliquot/50 mL of 2 × L-15.

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36 After 1 h incubation of MDCK cells with sample: Add **200 µl trypsin** to each 50 mL tube of 2 × L-15.

37 Make overlay media by adding **100 mL 2 × L-15 + trypsinsolution** to **100 mL 1.8% agarose** and mix well (*see* **Note 27**).

38 Add **3 mL overlay medium** /well and leave at **Room temperature** until it sets.

39 

Incubate upside-down at **37 °C** , **5 % CO2** for 3 days. Plaques maybe visible by the end of day 2 and the plates can be incubated till day 4 if plaques are too small on day 3.

40 

Count the plaques. This can be done by holding the plates against the light. Alternatively, remove agarose overlay and stain with crystal violet. To stain, cover the cells with a minimal amount of crystal violet solution for ~ **00:15:00** . Rock plates if necessary to ensure even coverage. Gently wash off the crystal violet stain with water. Once fixed, stained, and dried, store plaques indefinitely for future analysis.

41 

Calculate viral titer in PFU/mL: Average count of duplicate well × Dilution factor × (1000/150) = PFU/mL (multiply lung homogenate counts by 2 to give total viral load, as lungs were taken and homogenized in **2 mL RPMI**).

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