Mucosal associated invariant T cells: a new player in antibacterial immunity

James Ussher
“Infectious diseases made the largest contribution to hospital admissions of any cause”

“increased from 20.5% of acute admissions in 1989-93, to 26.6% in 2004-2008”

“clear ethnic and social inequalities in infectious disease risk”
The growing problem of antimicrobial resistance
“The problem is so serious that it threatens the achievements of modern medicine. A post-antibiotic era— in which common infections and minor injuries can kill—is a very real possibility for the 21st century.”
Mucosal surfaces – the central battleground

• Of the top 10 causes of infectious mortality worldwide, 9 are primarily transmitted via a mucosal route – >11 million deaths annually
• Commensal flora
• Constant environmental exposure to pathogens
Mucosal Associated Invariant T (MAIT) cells

- Abundant “innate-like” T cell population
- Found at mucosal surfaces
- Enriched in liver
- In blood, ~10% of CD8+ T cell population
  - ~100x more common than iNKT cells
- Rare in mice
- Semi-invariant TCR (Vα7.2-Jα33)
- Restricted by MHC related protein 1 (MR1)
  - Non-classical MHC class Ib protein
  - Non-polymorphic
  - Evolutionarily conserved
- Phenotype
  - Effector memory
  - CCR2+, CCR5+, CXCR6+
  - CD161++ IL23R+ CCR6+ RORγt+
  - IL17, IL22, IFNγ, TNFα
Specific activation of MAIT cells by bacteria

Activated by:
- *Escherichia coli*
- *Klebsiella pneumoniae*
- *Salmonella spp.*
- *Pseudomonas aeruginosa*
- *Francisella tularensis*
- *Staphylococcus aureus*
- *Staphylococcus epidermidis*
- *Mycobacterium tuberculosis*
- *Mycobacterium abscessus*
- *Lactobacillus acidophilus*
- *Candida albicans*
- *Candida glabrata*
- *Saccharomyces cerevisiae*

Not activated by:
- *Streptococcus pyogenes*
- *Enterococcus faecalis*
MR1 binds vitamin B metabolites

- Activating ligand in supernatant of *Salmonella* sp. culture
- Intermediate of riboflavin biosynthesis


5-Amino-6-d-ribitylaminouracil + R₁R₂ → Pyrimidines → Lumazines
MAIT cells protect against bacterial infection in vivo: mice

Wild type vs MR1\(^{-/-}\) mice

Intraperitoneal injection of luminescent *Klebsiella pneumoniae*  
CFU in spleen post aerosol challenge with BCG

Georgel et al. Mol Immunol 2011  
Chua et al, Infect Immun 2012
MAIT cells protect against bacterial infection \textit{in vivo}: humans
Persistent loss of MAIT cells from the blood in HIV despite HAART

HC = Healthy control

Cosgrove, Ussher et al, Blood 2013
• Activated by a wide range of bacteria
• Probable frequent exposure
  – Ligand is soluble and present in bacterial culture supernatant
• Inappropriate activation could cause immunopathology
How is MAIT cell activation regulated?

- Activated by a wide range of bacteria
- Probable frequent exposure
  - Ligand is soluble and present in bacterial culture supernatant
- Inappropriate activation could cause immunopathology
Cytokine-dependent activation of MAIT cells: expression of IL-18 receptor

Cytokine-dependent activation of MAIT cells: IL-12+IL-18 specifically induces IFNγ

PBMCs, overnight incubation

How do IL-12+IL-18 contribute to MAIT cell activation in bacterial infection?

20 hours

Fixed *E. coli* + THP-1 + Brefeldin A

16 hours

CD8+ T Cells

4 hours

IFN-γ

Gated on CD3+CD8+ lymphocytes
20 hours

Fixed *E. coli*  THP-1  +  Brefeldin A

CD8+ T Cells

16 hours  4 hours

IFN-γ+CD8+CD161++  T Cells
20 hours

5 hours

20 hours

5 hours
Whole bacteria, but not supernatant or cell lysate, are potent activators of MAIT cells.
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Activation by intact *E. coli* inhibited by:
- Cytochalasin D
- Bafilomycin A
Is surface expression of MR1 limiting?

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MR1 surface expression in THP1s is tightly regulated, even when over expressed.
Small increase in MR1 surface expression after treatment with *E. coli*

- THP1
- THP1.hMR1

- % of Max
- B1-A: MR1/Iso AF488-A

- Isotype
- Untreated
- *E. coli* 6 hours
- *E. coli* overnight

Surface antibody stain
Increased trafficking of MR1 to the cell surface after *E. coli* treatment

**THP1**

**THP1.hMR1**

Fluorescently labeled αMR1 added to culture for final 4 hours

**Antibody capture assay**

- **Isotype**
  - Untreated
  - *E. coli* 6 hours
  - *E. coli* overnight
Activation induces trafficking of MR1 to the cell surface

+ TLR2 agonist

4 hour antibody capture assay
NFκB signaling is required for activation-induced trafficking of MR1 to the cell surface.
Time of APC exposure to bacteria

**Overnight**

Fixed *E. coli* OR Supernatant + THP-1

17 hours + CD8+ T Cells

Brefeldin A

1 hour + IFN-γ+CD8+CD161++ T Cells

**7 hours**

THP-1

15 hours + 2 hours + CD8+ T Cells

Brefeldin A

1 hour + 4 hours + IFN-γ+CD8+CD161++ T Cells
Supernatant: MR1 over-expression but not incubation time enhances MAIT cell activation.
Supernatant: MR1 over-expression but not incubation time enhances MAIT cell activation.

MR1 limiting
No increase with time
Intact *E. coli*: Prolonged incubation but not MR1 expression enhances MAIT cell activation.
Intact *E. coli*: Prolonged incubation but not MR1 expression enhances MAIT cell activation

MR1 not limiting
Time-dependent process
Intact bacteria remain more potent
NFκB signaling in the APC is required for MR1-mediated MAIT cell activation
Effect of pre-activation of THP1s on early MR1-mediated MAIT cell activation
Pre-activation of THP1s with agonists to TLRs 1, 2, or 6 enhances early MR1-mediated MAIT cell activation.
Pre-treatment with IFN$\gamma$ or IFN$\alpha$ also enhances early MR1-mediated MAIT cell activation.

+/- interferon-$\gamma$ 1000U/ml overnight

+/- interferon-$\alpha$ 1000U/ml overnight
NFκB signaling in monocyte-derived macrophages (MoMφ) is required for MAIT cell activation.

Monocyte-derived macrophages + E. coli ON
Robust early MR1-mediated activation with MoMφ

Monocyte-derived macrophages
Pre-activation of MoMϕ fails to enhance MR1-mediated MAIT cell activation

Monocyte-derived macrophages
MR1-mediated MAIT cell activation is negatively regulated by endotoxin tolerance
Summary (1)

• Efficient MR1-mediated activation requires APC activation
• LPS-induced tolerance suppresses MR1-mediated activation
• MR1-mediated MAIT cell activation is tightly regulated
Model of regulation of MAIT cell activation

IFNγ + TNFα + IL17A

MR1-dependent

MAIT cell

TCR(Vα7.2)

MR1

APC
Model of regulation of MAIT cell activation

IFNγ + TNFα + IL17A

MR1-dependent

MAIT cell

TCR(\text{V_\alpha 7.2})

\uparrow \text{MR1}

APC

OR

TLR

IFNγR

NFκB

MR1-dependent

IFN\gamma + TNF\alpha + IL17A

TCR(V_\alpha 7.2)

\uparrow \text{MR1}

APC

OR

TLR

IFN\gamma R

NFκB
Model of regulation of MAIT cell activation

IFNγ + TNFα + IL17A

MR1-dependent

Pre-exposure to LPS

TCR(Vα7.2)

MR1

NFκB

APC

TLR
Model of regulation of MAIT cell activation

IL12 + IL18

IFNγ

IL12R

IL18R

MAIT cell

NFκB

TLR

APC

IL12+IL18-dependent Late
Model of regulation of MAIT cell activation

Non-riboflavin-producing bacteria and other inflammatory stimuli

IL12+IL18-dependent

Late

IFNγ

IL12R

IL18R

MAIT cell

TLR

NFκB

APC
Summary (2)

• Two mechanisms of activation:
  – MR1 (TCR dependent)
  – IL-12+IL-18 (TCR independent)

• Whole bacteria, but not supernatant or cell lysate, are potent activators of MAIT cells via MR1
  – Presentation of supernatant dependent upon surface expression of MR1
  – Presentation of intact bacteria dependent upon time but not level of MR1 expression

• Efficient MR1-mediated activation requires APC activation
  – NFκB-dependent

• LPS-induced tolerance suppresses MR1-mediated activation

• MR1-mediated MAIT cell activation is tightly regulated
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Is the MAIT cell population perturbed in HIV infection?
MAIT cells in HIV infection

- Early stage HIV infection
  - SPARTAC baseline samples
  - Median CD4 count = 603 cells/µl (475, 774)
  - Median viral load = $4.73 \log_{10}$ copies/ml (3.89, 5.19)

- Chronic untreated HIV infection
  - Kings College London Infectious Diseases Biobank
  - Median CD4 count = 250 cells/µl (207, 326)
  - Median viral load = $4.22 \log_{10}$ copies/ml (3.99, 4.94)

Cosgrove, Ussher et al, Blood 2013
MAIT cells are lost from the blood in HIV

HC = Healthy control

Cosgrove, Ussher et al, Blood 2013
MAIT cells are lost from the blood in HIV

Canonical TCR Vα7.2-Jα33
Normalised against Cα

Unpublished data
MAIT cells are not enriched in colon in HIV

12 HIV+ patients
Macroscopically normal colon
7 microscopic colitis

CD3+CD8+MDR1++

Healthy control

12 age-matched controls
Non-inflamed normal colon

Cosgrove, Ussher et al, Blood 2013
MAIT cells are not enriched in colon in HIV

HC = Healthy control

Cosgrove, Ussher et al, Blood 2013
MAIT cells do not recover with HAART

- Swiss HIV cohort study
  - 30 patients
  - Pre-HAART and 1 and 2 years on HAART
  - Fully suppressed viral load

Cosgrove, Ussher et al, Blood 2013
What is the mechanism of MAIT cell loss in HIV infection?
HIV does not preferentially infect MAIT cells

- PBMCs from healthy subjects
- Activated for 3 days
  - PHA, IL2 and IL7
- Infected with HIV at MOI 10
  - CCR5-tropic virus (JR-CSF)
  - CXCR4 tropic virus (MN)
- p24 detected at days 6 and 9

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Cosgrove, Ussher et al, Blood 2013
Bacterial lipopolysaccharide is detectable in the lamina propria in HIV infection

Cosgrove, Ussher et al, Blood 2013
*E. coli* induces apoptosis of MAIT cells *in vitro*

Cosgrove, Ussher et al, Blood 2013
Blocking MR1 inhibits *E. coli*-induced apoptosis

Cosgrove, Ussher et al, Blood 2013
Proposed model for MAIT cell depletion in HIV infection
Proposed model for MAIT cell depletion in HIV infection

Circulating blood

Lamina propria

Gut Lumen

MAIT

MAIT

MAIT

MAIT

APC

E. coli
Proposed model for MAIT cell depletion in HIV infection

Circulating blood → Lamina propria → Gut Lumen

MAIT cells are depicted moving from circulating blood to the lamina propria, where they are influenced by chemokines and APCs. The gut lumen contains E. coli, which may contribute to MAIT cell depletion.
Proposed model for MAIT cell depletion in HIV infection

Circulating blood

Lamina propria

Gut Lumen

E. coli

MAIT

APC

MAIT

MAIT

MAIT

MAIT

MAIT

MAIT

MAIT

MAIT

MAIT

MAIT
Proposed model for MAIT cell depletion in HIV infection

Circulating blood

Lamina propria

Gut Lumen

APC

MAIT

E. coli
Summary (1)

• MAIT cells are lost from the blood early in HIV infection
• Not enriched in the colon
• Fail to recover with HAART
• Activation induced cell death potential mechanism of loss
  – Evidence of microbial translocation \textit{in vivo}
  – MR1-dependent cell death \textit{in vitro} following activation by 
    \textit{E. coli}
• Potential implications for control of bacterial infections
  – \textit{Mycobacterium tuberculosis}
  – Non-typhoidal \textit{Salmonella} spp.
  – Invasive pneumococcal disease
• Reconstitution potential therapeutic target