T CELLS

Copyright © 2019 The Authors, some rights reserved; exclusive licensee American Association for the Advancement of Science. No claim to original U.S. Government Works

IL-23 costimulates antigen-specific MAIT cell activation and enables vaccination against bacterial infection

Huimeng Wang[1,2], Lars Kjer-Nielsen[1], Mai Shi[1,3], Criselle D'Souza[1,4], Troi J. Pediongco[1], Hanwei Cao[1], Lyudmila Kostenko[1], Xin Yi Lim[1], Sidonia B. G. Eckle[1], Bronwyn S. Meehan[1], Tianyuan Zhu[1,3], Bingjie Wang[1], Zhe Zhao[1], Jeffrey Y. W. Mak[5,6], David P. Fairlie[5,6], Michele W. L. Teng[7], Jamie Rossjohn[8,9,10], Di Yu[11], Barbara Fazekas de St Groth[12], George Lovrecz[13], Louis Lu[13], James McCluskey[1], Richard A. Strugnell[1]*†, Alexandra J. Corbett[1]*†, Zhenjun Chen[1]*†

Mucosal-associated invariant T (MAIT) cells are activated in a TCR-dependent manner by antigens derived from the riboflavin synthesis pathway, including 5-(2-oxopropylideneamino)-6-D-ribitylaminouracil (5-OP-RU), bound to MHC-related protein-1 (MR1). However, MAIT cell activation in vivo has not been studied in detail. Here, we have found and characterized additional molecular signals required for optimal activation and expansion of MAIT cells after pulmonary *Legionella* or *Salmonella* infection in mice. We show that either bone marrow–derived APCs or non–bone marrow–derived cells can activate MAIT cells in vivo, depending on the pathogen. Optimal MAIT cell activation in vivo requires signaling through the inducible T cell costimulator (ICOS), which is highly expressed on MAIT cells. Subsequent expansion and maintenance of MAIT-17/1-type responses are dependent on IL-23. Vaccination with IL-23 plus 5-OP-RU augments MAIT cell–mediated control of pulmonary *Legionella* infection. These findings reveal cellular and molecular targets for manipulating MAIT cell function under physiological conditions.

Downloaded from http://immunology.sciencemag.org/ by guest on January 25, 2021

INTRODUCTION

Mucosal-associated invariant T (MAIT) cells respond to a range of bacteria and fungi by recognizing compounds derived from riboflavin biosynthesis, the most potent of which is 5-(2-oxopropylideneamino)-6-D-ribitylaminouracil (5-OP-RU), presented by major histocompatibility complex–related protein-1 (MR1) (*1*, *2*). MAIT cells express a conserved αβ T cell receptor (TCRαβ), consisting of a relatively invariant TCRα chain coupled with a broad repertoire of β chains, but with preferential Vβ usage (*3*–*7*). In mice, MAIT TCRs use TCR alpha variable gene segment (TRAV)1-TRAJ33 (Vα19-Jα33) generally assembled with TCR beta variable gene segment (TRBV)19 (Vβ6) or TRBV13 (Vβ8), and homologous TCRs exist in humans. MAIT cells have been shown to respond to a wide range of bacteria and fungi, including both pathogenic and commensal species, correlating with the presence of genes involved in riboflavin biosynthesis in these organisms and thus with their capacity for antigen (Ag) production (*2*, *8*–*10*). Multiple studies suggest that MAIT cells have evolved for host defense against microbial pathogens. However, MR1 is ubiquitously expressed at low levels (*11*–*13*), and the cells responsible for presenting Ag via MR1 to MAIT cells during bona fide infection have not been described. Moreover, the requirements for activation of MAIT cells and their role in protecting against pathogens are still being unraveled.

MAIT cells that egress from the thymus display memory/effector cell properties (*14*–*17*), making them distinct from naïve conventional T cells. However, after TCR-dependent stimulation, MAIT cells acquire an activated status, allowing them to expand and rapidly produce cytokines, including interleukin-17 (IL-17), interferon-γ (IFN-γ), tumor necrosis factor–α (TNF-α), and granulocyte-macrophage colony-stimulating factor in an "innate-like" manner in response to bacteria (*15*, *18*). Recently, we demonstrated that MAIT cells differ from conventional circulating cells as they appear to be activated to proliferate at the site of infection, contributing substantially to the accumulation of MAIT cells at these sites (*19*, *20*). Similar to conventional T cells, MAIT cells also require other costimulatory signals in addition to TCR recognition of synthetic 5-OP-RU antigen (Ag) for their activation (*19*, *21*). These additional signals can be provided by Toll-like receptor (TLR) agonists or bacterial products (*19*), but the nature of such obligatory signals for MAIT cell priming in vivo is not well understood. Certain cytokines are known to be important in TCR-dependent activation of MAIT cells (*22*–*25*), with IL-12 and IL-18, in particular, having been reported to drive MAIT cell activation independently of MR1-TCR ligation in in vitro assays (*8*, *26*).

Either activation of MAIT cells through prior vaccination with 5-OP-RU in the presence of TLR agonists or adoptive transfer of activated MAIT cells can confer improved protection against bacterial infection (*20*). To understand the signals required to stimulate MAIT cells for optimal function in vivo, we embarked on a

[1]Department of Microbiology and Immunology, University of Melbourne, Peter Doherty Institute for Infection and Immunity, Melbourne, VIC 3000, Australia. [2]State Key Laboratory of Respiratory Disease, Guangzhou Institute of Respiratory Disease, The First Affiliated Hospital of Guangzhou Medical University, Guangzhou, Guangdong 510182, China. [3]School of Medicine, Tsinghua University, Beijing, China. [4]Sir Peter MacCallum Department of Oncology, University of Melbourne, VIC 3010, Australia. [5]Division of Chemistry and Structural Biology, Institute for Molecular Bioscience, University of Queensland, Saint Lucia, QLD 4072, Australia. [6]Australian Research Council Centre of Excellence in Advanced Molecular Imaging, University of Queensland, Saint Lucia, QLD 4072, Australia. [7]QIMR Berghofer Medical Research Institute, Herston, QLD 4006, Australia. [8]Infection and Immunity Program and the Department of Biochemistry and Molecular Biology, Biomedicine Discovery Institute, Monash University, Clayton, VIC 3800, Australia. [9]Australian Research Council Centre of Excellence in Advanced Molecular Imaging, Monash University, Clayton, VIC 3800, Australia. [10]Institute of Infection and Immunity, Cardiff University, School of Medicine, Heath Park, CF14 4XN Wales, UK. [11]John Curtin School of Medical Research, The Australian National University, Acton, ACT 2601 Australia. [12]Discipline of Pathology, Charles Perkins Centre, Faculty of Medicine and Health, University of Sydney, Sydney, NSW 2006, Australia. [13]Biomedical Manufacturing, CSIRO, Parkville, VIC, 3052, Australia.
*These authors contributed equally to this work.
†Corresponding author. Email: zhenjun@unimelb.edu.au (Z.C.); corbetta@unimelb.edu.au (A.J.C.); rastru@unimelb.edu.au (R.A.S.)

study of their activation requirements in mouse models of infection with two pathogens, *Legionella longbeachae* and *Salmonella enterica* var Typhimurium (*S.* Typhimurium). Here, we use bone marrow (BM) chimeras, in which either BM-derived [wild type (WT) → $Mr1^{-/-}$] or non–BM-derived ($Mr1^{-/-}$ → WT) cells, or both (WT → WT), express MR1 and hence could potentially present Ag through MR1 to show that either BM-derived antigen-presenting cells (APCs) or non–BM-derived cells can activate MAIT cells in vivo depending on the pathogen. Inducible T cell costimulator (ICOS) is highly expressed on MAIT cells and is required for optimal MAIT cell activation in vivo. We demonstrate that subsequent expansion and maintenance of MAIT-17/1-type responses depend on IL-23 and that combined vaccination of mice with IL-23 and 5-OP-RU augments MAIT cell–mediated control of pulmonary *Legionella* infection.

RESULTS

Differential requirement for BM- and non–BM-derived cells to activate MAIT cells via MR1, depending on the biology of the bacterial infection

The low-level, ubiquitous expression of MR1 (*11*–*13*) has made it difficult to identify the cells that drive MAIT cell responses in vivo. Studies reporting MAIT cell activation in vitro have used both BM- and non–BM-derived cells or cell lines (*23*, *27*–*29*), suggesting that either cell type is capable of presenting Ag on MR1 for MAIT cell activation. To assess the ability of different MR1-expressing cells that are BM-derived or not to activate and drive proliferation of MAIT cells in vivo in an MR1-dependent manner, we used two models of intranasal infection of C57BL/6 mice with *S.* Typhimurium BRD509 or *L. longbeachae* NSW150, in which we have previously reported activation and expansion of MAIT cells (*19*, *20*). We first confirmed that MAIT cells accumulated in the infected site (lungs) during pulmonary infection. Seven days after intranasal infection with *S.* Typhimurium BRD509 or *L. longbeachae* NSW150, MAIT cells represented, on average, 46 and 24%, respectively, of TCRβ$^+$ lymphocytes in the lungs as detected with MR1–5-OP-RU tetramers (Fig. 1A, gating strategy shown in fig. S1A). Although the MAIT cells observed in infected lungs could derive from contaminating MAIT cells from the circulation (*30*), this is not a plausible explanation considering the low numbers of MAIT cells in the circulation of mice (<0.1% of T cells) (*19*, *31*) and the documented capacity for MAIT cell proliferation in the lungs after infection (*20*). Hence, we regard circulating MAIT cells as only

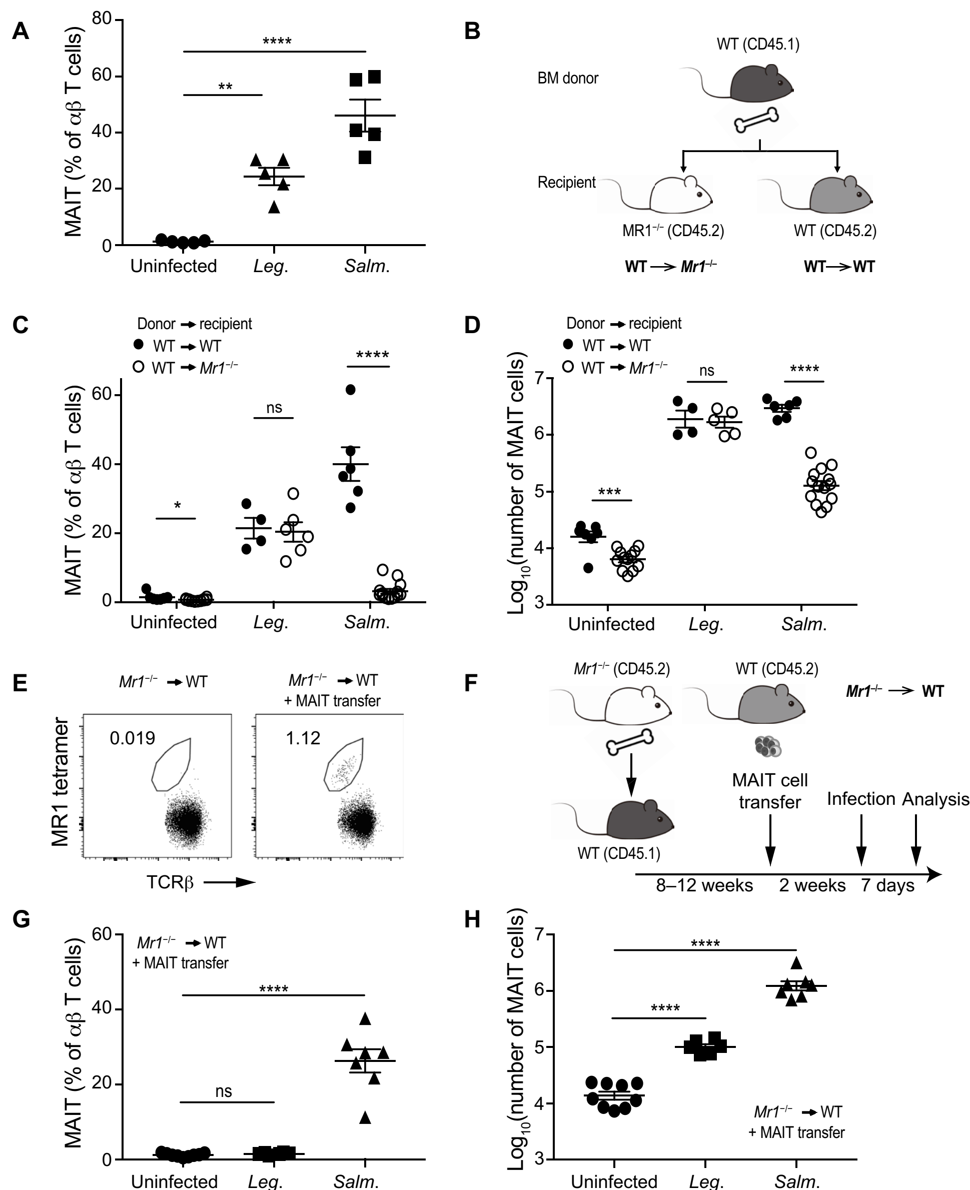

Fig. 1. Both BM-derived and non–BM-derived APCs can drive MAIT cell accumulation in the lungs in response to bacterial infection. (**A**) MAIT cell frequency in the lungs of C57BL/6 mice uninfected or at day 7 after intranasal infection with 10^6 *S.* Typhimurium BRD509 (*Salm.*) or 10^4 *L. longbeachae* (*Leg.*). Data show individual mice and means ± SEM. ***P* < 0.01, *****P* < 0.0001, one-way ANOVA with Dunnett's multiple comparisons. (**B**) Schematic of BM chimeras for (C) and (D). (**C**) Frequency and (**D**) absolute number of MAIT cells of WT → WT or WT → $Mr1^{-/-}$ chimeric mice uninfected or on day 7 p.i. intranasally infected with 10^6 *S.* Typhimurium BRD509 or 10^4 *L. longbeachae*. **P* < 0.05, ****P* < 0.001, *****P* < 0.0001, multiple unpaired *t* test. (**E**) Lung CD45.2$^+$TCRβ$^+$ cells from $Mr1^{-/-}$ → WT chimeric mice with or without 10^5 adoptively transferred C57BL/6 MAIT cells 2 weeks after transfer showing reconstitution of MAIT cells in the lungs. (**F**) Schematic of protocol and BM chimeras for (G) and (H). (**G**) Frequency and (**H**) absolute number of transferred MAIT cells on day 7 p.i. in $Mr1^{-/-}$ → WT chimeric mice, adoptively transferred with 10^5 WT MAIT cells and then intranasally infected with 10^6 *S.* Typhimurium BRD509 or 10^4 *L. longbeachae*. ns, nonsignificant. *****P* < 0.0001, one-way ANOVA with Dunnett's multiple comparisons. Also see fig. S1.

Downloaded from http://immunology.sciencemag.org/ by guest on January 25, 2021

minor contributors to the expansion of MAIT cells. To determine the source of APC required to present bacterial Ag via MR1 to MAIT cells in these models, we generated three sets of BM chimeric mice: (i) WT C57BL/6 (CD45.1) BM transplanted into recipient MR1-deficient (CD45.2) mice, (ii) WT C57BL/6 (CD45.1) BM transplanted into recipient WT congenic (CD45.2) mice, or (iii) MR1-deficient BM ($Mr1^{-/-}$, CD45.2) transplanted into recipient WT C57BL/6 mice (CD45.1). These BM transfers resulted in chimeric mice that could present Ag to MAIT cells only via BM-derived cells (WT → $Mr1^{-/-}$), via both BM- and non–BM-derived cells (WT → WT) (Fig. 1B) or only via non–BM-derived cells ($Mr1^{-/-}$ → WT) (Fig. 1F). Congenic markers (CD45.1 or CD45.2) were used to track the donor- and recipient-derived cells in these mice. We validated reconstitution of cells in the lungs of chimeric mice 8 weeks later, where >99% of $CD3^{-}TCR\beta^{-}CD45^{+}$ cells were derived from donor BM (fig. S1B).

BM chimeric mice were then infected intranasally with either *S.* Typhimurium or *L. longbeachae*, and MAIT cells from the lungs were examined on day 7 post infection (p.i.). After *L. longbeachae* infection, MAIT cells expanded similarly in WT → $Mr1^{-/-}$ mice and WT → WT controls (percentage of αβ T cells or absolute number of MAIT cells) (Fig. 1, C and D), suggesting that MR1 expression on BM-derived APCs is sufficient for activation of MAIT cells in this infection model. However, in mice infected with *S.* Typhimurium BRD509, there were significantly fewer MAIT cells in the lungs of WT → $Mr1^{-/-}$ chimeric mice on day 7 p.i. compared with WT → WT controls (Fig. 1, C and D), indicating that expression of MR1 on BM-derived cells is insufficient for complete MAIT cell activation in response to *S.* Typhimurium pulmonary infection. There were no significant differences in the non-MAIT T cell response to either bacterial infection in WT → $Mr1^{-/-}$ versus WT → WT BM chimeras (fig. S1C).

To determine whether non–BM-derived cells could provide APC function for MAIT cell activation, we created ($Mr1^{-/-}$ → WT) BM chimeric mice. However, no donor MAIT cells were detected in the lungs of these mice (Fig. 1E), consistent with the requirement for $MR1^{+}$ double positive (DP) thymocytes in MAIT cell development (*32*). Hence, to examine MAIT cells in this setting, where only non–BM-derived cells expressed MR1, we adoptively transferred MAIT cells sorted from the lungs of *S.* Typhimurium BRD509–infected WT (CD45.2) mice (Fig. 1F). This transfer reconstituted MAIT cells to a similar frequency as compared with naïve C57BL/6 mice 2 weeks after transfer (Fig. 1E). We then infected these mice intranasally with *S.* Typhimurium BRD509 or *L. longbeachae* NSW150. In *S.* Typhimurium–infected mice, we found a selective enrichment of transferred MAIT cells in the lungs: proportionally expanding from ~1 to ~30% of all αβ T cells (Fig. 1G) with an ~90-fold increase in the absolute number of MAIT cells (Fig. 1H). In contrast, after *L. longbeachae* infection, no significant increase in the proportion of MAIT cells was observed (Fig. 1G), and the expansion of absolute MAIT cell numbers was limited (~6-fold) (Fig. 1H). To further validate the use of transferred MAIT cells in this model, we transferred MAIT cells (bearing distinct allotypes of CD45 from endogenous MAIT cells) into WT → $Mr1^{-/-}$ chimeras. These mice were then infected with either *S.* Typhimurium or *L. longbeachae* 2 weeks after transfer and examined for the accumulation of transferred and endogenous MAIT cells using CD45 congenic markers at 7 days p.i. The transferred MAIT cells expanded with a similar pattern to endogenous MAIT cells (Fig. 1D versus fig. S1D) after infection. Together, these data suggest that MR1 expressing non–BM-derived cells could activate MAIT cells in response to *S.* Typhimurium BRD509, but not *L. longbeachae* infection, which required MR1 expression on BM-derived cells for MAIT cell expansion.

Active invasion by *S.* Typhimurium is essential for its capacity to activate MAIT cells in vivo

Although priming and activation of conventional T cells by epithelial cells presenting peptide Ag have been demonstrated by others (*33*, *34*), it was unexpected that non–BM-derived cells appeared to be dominant in driving MAIT responses to *S.* Typhimurium infection. To understand why non–BM-derived cells are required for the accumulation of MAIT cells during *S.* Typhimurium pulmonary infection, we used the *S.* Typhimurium SL1344Δ*invA* mutant, which lacks the ability to actively invade epithelial cells (*35*). Presentation of Ag in this infection is largely restricted to professional APCs that are capable of phagocytosing the bacteria (*36*). First, we tested the SL1344Δ*invA* mutant in an MR1-dependent in vitro activation assay (*37*) to confirm its ability to produce MAIT stimulatory Ag. Bacterial culture supernatants from *S.* Typhimurium SL1344Δ*invA* and SL1344 were equally capable of activating a reporter cell line expressing a MAIT TCR; Jurkat.MAIT (*2*, *37*), as detected by up-regulation of CD69 (Fig. 2A), suggesting no defect in MAIT cell Ag production by the SL1344Δ*invA* mutant. The defect in invasion of the SL1344Δ*invA* mutant (*35*) was confirmed in both human and murine macrophage versus epithelial cell lines. Thus, after 1-hour coculture with bacteria, we found that both human and murine epithelial cells contained significantly fewer SL1344Δ*invA* mutant colony-forming units (CFU) compared with the SL1344 control, whereas no difference in bacterial load per cell was observed in macrophage cell lines, which actively phagocytose bacteria (Fig. 2B).

To test the effect of this invasion defect on the accumulation of MAIT cells in vivo, we intranasally infected C57BL/6 mice with either SL1344 or SL1344Δ*invA* bacteria and examined MAIT cells from the lungs at day 6 p.i. In addition, we determined the bacterial load in these mice by CFU assay from homogenized lungs. In contrast to SL1344, the SL1344Δ*invA* mutant strain failed to evoke substantial MAIT cell accumulation in the lungs of these infected mice (Fig. 2C), despite having equivalent bacterial loads (Fig. 2D). Together, these data demonstrate that active bacterial invasion is essential to *S.* Typhimurium stimulation of an optimal MAIT cell response in vivo consistent with our finding that non–BM-derived cells are important APCs presenting Ag to MAIT cells in infection by this facultative intracellular pathogen. Thus, together, these data demonstrate that, depending on the pathogen biology, either BM- or non–BM-derived cells are capable of acting as APCs and activating MAIT cells in an MR1-dependent manner.

ICOS is highly expressed by MAIT cells and is critical for optimal MAIT cell expansion and retinoic acid–related orphan receptor γt (RORγt) expression

Previously, we showed that synthetic 5-OP-RU antigen was insufficient for MAIT cell activation in vivo and that other required stimulatory signals could be provided through co-inoculation of mice with TLR agonists CpG, polyinosinic:polycytidylic acid, or Pam2Cys (*19*). Here, we investigated the direct requirements for costimulatory molecules (signal 2) for MAIT cell activation, hypothesizing that TLR agonists may not directly activate MAIT cells. Rather, they might act indirectly through APCs, either by enhancing MR1 presentation (*38*) or

Downloaded from http://immunology.sciencemag.org/ by guest on January 25, 2021

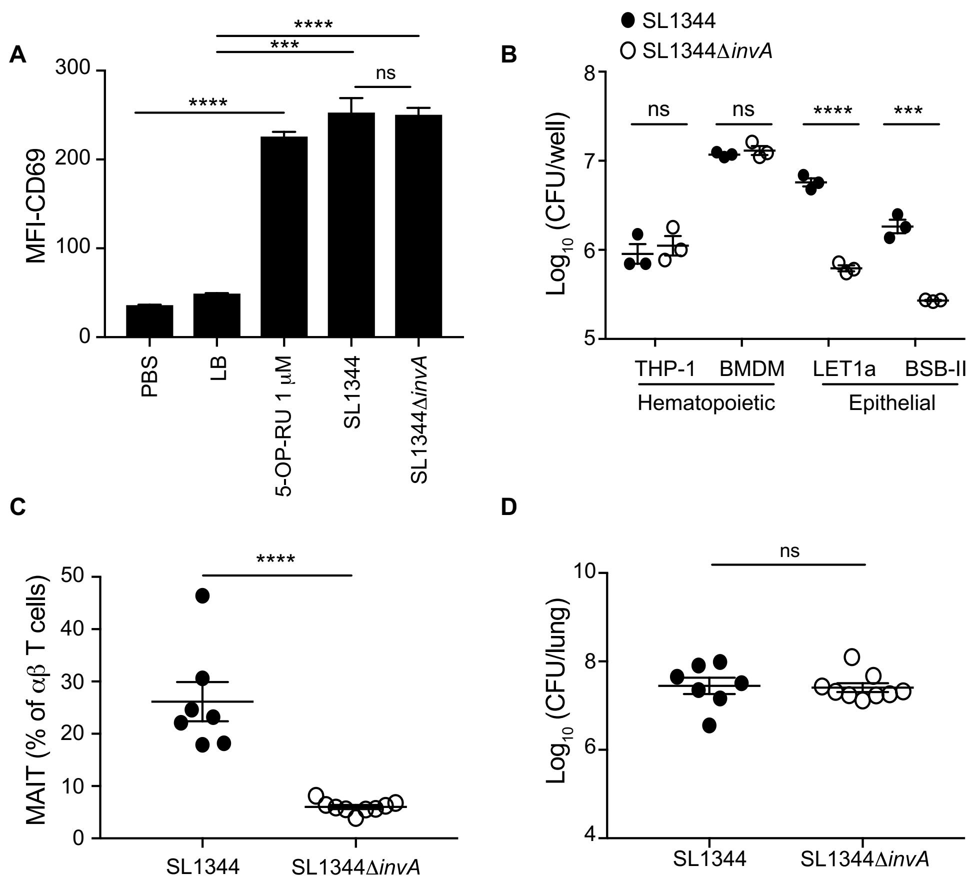

Fig. 2. Active invasion by *S.* Typhimurium is vital for MAIT cell stimulation in vivo. (**A**) CD69 expression on Jurkat.MAIT cells after coculture (for 16 hours) with C1R.MR1 cells and 5-OP-RU or filtered culture supernatant from *S.* Typhimurium SL1344, SL1344Δ*invA*, or media control (LB). Data show mean fluorescence intensity (MFI) of gated Jurkat.MAIT cells, with SEM of triplicate samples as error bars. $***P < 0.001$, $****P < 0.0001$, one-way ANOVA with Tukey's multiple comparisons. The experiment was performed twice with similar results. (**B**) Bacterial counts from human and murine cells (10^6 per well) of either epithelial [human: BSB-II; mouse: lung epithelial type 1 (LET1) (*96*)] or macrophage [human: THP-1; mouse: bone marrow–derived macrophages (BMDM)] origin after 1-hour infection with SL1344 (open circle) or SL1344Δ*invA* (closed circle) *Salmonella* (MOI, 10). (**C** and **D**) MAIT cells as a percentage of αβ T cells (C) and bacterial load (CFU) (D) from the lungs on day 6 p.i. of C57BL/6 mice intranasally infected with 10^5 *S.* Typhimurium SL1344 or SL1344Δ*invA*. Pooled data show means ± SEM of seven to nine mice per group. $***P < 0.001$, $****P < 0.0001$, unpaired *t* test. The experiments were carried out independently two times with similar results.

by stimulating cytokine production or up-regulation of costimulatory ligands. First, we examined the expression of a range of costimulatory molecules on MAIT and non-MAIT TCRβ$^+$ cells from the lungs of naïve C57BL/6 WT mice. ICOS was highly expressed by MAIT cells from naïve mice, compared with non-MAIT T cells (Fig. 3A). In addition, MAIT cells expressed intermediate levels of CD154 (CD40L) and CD27 and similar levels of CD28 to non-MAIT T cells, whereas CD137 (4-1BB) was not detected on lung MAIT cells. Thus, we hypothesized that ICOS could play an important role in the costimulation of MAIT cells.

To further investigate whether these costimulatory molecules were required for optimal accumulation and activation of MAIT cells during bacterial infection, we studied gene knockout (KO) mice (*Icos*$^{-/-}$ mice and *Cd80*$^{-/-}$/*Cd86*$^{-/-}$ double KO mice; the latter lack signaling through CD28). WT (C57BL/6), *Icos*$^{-/-}$, and *Cd80*$^{-/-}$/*Cd86*$^{-/-}$ double KO mice were intranasally infected with *S.* Typhimurium BRD509 or *L. longbeachae* NSW150 and then examined for MAIT cell and CD4$^+$ T cell accumulation in the lungs after 7 days (Fig. 3B and fig. S2, A and B). Compared with WT mice, we observed a significant reduction in the proportion and absolute number of MAIT cells responding to infection in *Icos*$^{-/-}$ mice for both *S.* Typhimurium and *L. longbeachae* infections. There was no significant difference in MAIT cell numbers detected between WT mice and *Cd80*$^{-/-}$/*Cd86*$^{-/-}$ mice (Fig. 3B and fig. S2, A and B) or in the number of CD4$^+$ T cells in WT versus *Icos*$^{-/-}$ mice after pulmonary infection with *S.* Typhimurium BRD509 or *L. longbeachae* NSW150 (fig. S2, A and B).

We next examined the expression of key transcription factors, T-bet and RORγt, which drive T helper 1 (T_H1)– and T_H17-type responses, respectively (*39*, *40*), by the responding MAIT cells, to determine whether different costimulatory signals may drive phenotypic differences in MAIT cells. We have previously observed an increase in T-bet expression on lung MAIT cells after infection with *S.* Typhimurium or *L. longbeachae* (*19*, *20*). Consistent with our previous data, in WT C57BL/6 mice, there was an increase in the proportion of both T-bet$^+$RORγt$^+$ and T-bet$^+$RORγt$^-$ MAIT cells after infection (Fig. 3, C and D). A similar effect was observed in *Cd80*$^{-/-}$ *Cd86*$^{-/-}$ mice. In contrast, in the absence of ICOS, there was a reduction in the numbers of RORγt$^+$T-bet$^-$ MAIT cells after *S.* Typhimurium or *L. longbeachae* infection, and proportionally T-bet$^+$RORγt$^-$ MAIT cells were increased (Fig. 3D and fig. S2, C and D). These data suggest that ICOS is required for MAIT cell maintenance of RORγt expression and, in the absence of ICOS, more MAIT cells were skewed toward a T_H1-like phenotype (MAIT-1) with high expression of T-bet, presumably reflecting other signals in play.

IL-23 is required for optimal MAIT cell accumulation and activation during pulmonary bacterial infection

Optimal MAIT cell responses require additional signals concurrent with TCR Ag recognition (*19*). However, although previous studies have shown that cytokines, including IL-12 and IL-18, can act in a TCR-independent manner to stimulate MAIT cells (*8*), the requirement and type of cytokines that enhance MR1-dependent MAIT cell activation (*22*–*25*, *41*), particularly in vivo, are less well understood. To probe the cytokine requirements for MAIT cell activation in vivo, we intranasally infected mice genetically deficient in one or more individual cytokines with *S.* Typhimurium BRD509, and we analyzed MAIT cell accumulation in the lungs at day 7 p.i. Mice deficient in

Downloaded from http://immunology.sciencemag.org/ by guest on January 25, 2021

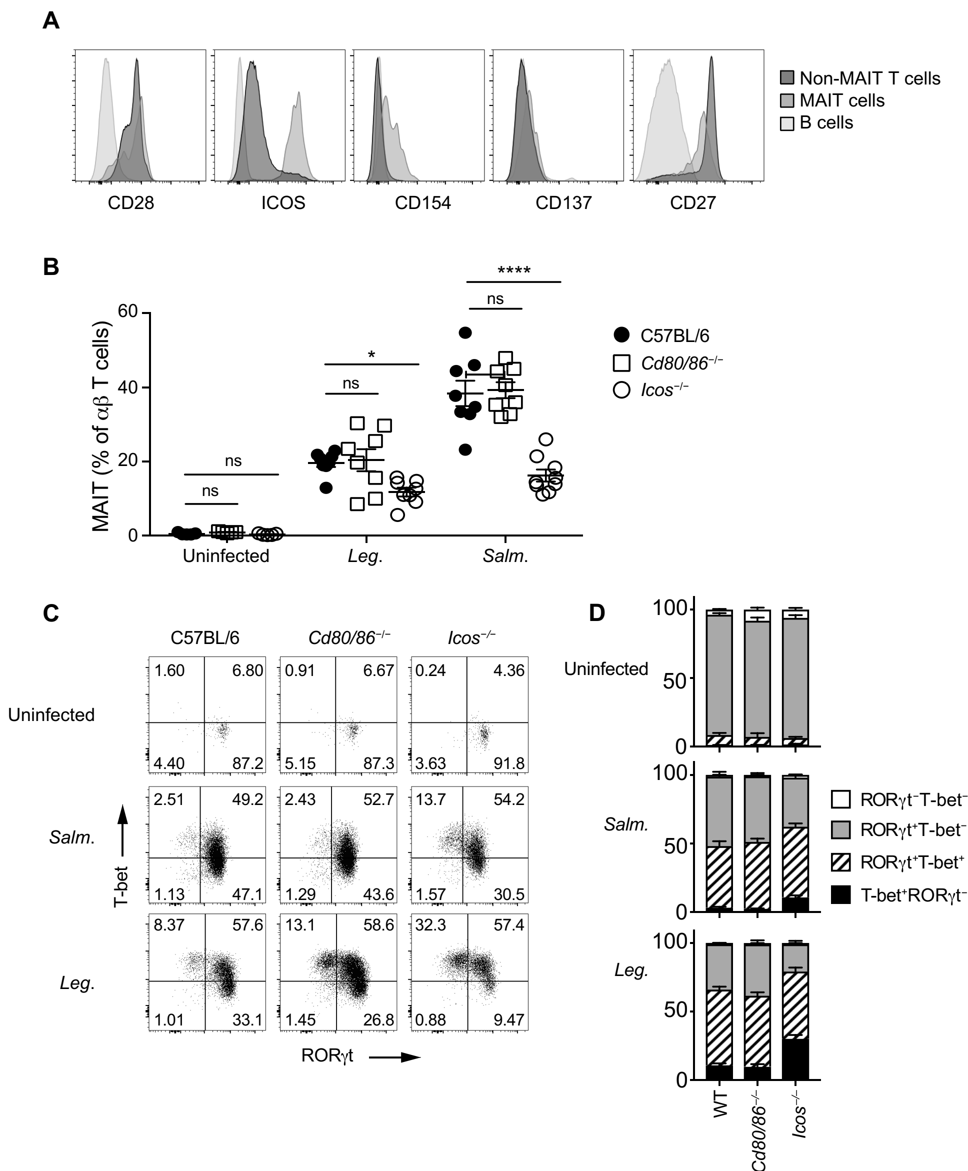

Fig. 3. ICOS is highly expressed on MAIT cells and is important for driving their response to infection. (**A**) Representative FACS histograms showing expression of CD154, CD28, CD137, CD27, and ICOS on MAIT cells, non-MAIT T cells, and B cells from the lungs of uninfected C57BL/6 WT mice. (**B**) MAIT cell frequency in the lungs at day 7 p.i. of *Cd80*$^{-/-}$*Cd86*$^{-/-}$, *Icos*$^{-/-}$, and C57BL/6 WT mice, intranasally infected with 10^6 *S.* Typhimurium BRD509 or 10^4 *L. longbeachae*. Data show individual mice and means ± SEM. *$P < 0.05$, ****$P < 0.0001$, one-way ANOVA with Dunnett's multiple comparisons. (**C**) Representative FACS plots and (**D**) stacked plots showing proportion of MAIT cells expressing T-bet and RORγt from naïve, *S.* Typhimurium BRD509–infected, or *L. longbeachae*–infected *Cd80*$^{-/-}$*Cd86*$^{-/-}$, *Icos*$^{-/-}$, and C57BL/6 WT mice (7 days p.i.), means ± SEM of five (naïve) or eight to nine (infected) mice, pooled from two experiments. See also fig. S2.

IL-18, IL-6, or IFN-γ, or *Il-12p35*$^{-/-}$ mice, which lack IL-12 and IL-35 (*42*), were all capable of mounting a robust MAIT cell response similar to WT mice (Fig. 4, A and B). In contrast, both *Il-12p40*$^{-/-}$ mice, which lack both IL-12 and IL-23, and *Il-23p19*$^{-/-}$ mice, which lack IL-23 (*42*–*45*), had a marked reduction in their MAIT cell accumulation (percentage of T cells and absolute number) in the lungs compared with WT mice (Fig. 4, A and B), indicating that the lack of IL-23 compromises the expansion of MAIT cells in the lungs in response to *S.* Typhimurium infection. A reduction in MAIT cell accumulation in *Il-23p19*$^{-/-}$ mice was also observed when mice were infected with *L. longbeachae* (fig. S3, A and B). In contrast, there was no significant difference in non-MAIT αβ T cell numbers in WT versus *Il-23p19*$^{-/-}$ mice (fig. S3C). In addition, we found no significant effect of either ICOS or IL-23 deficiency on numbers of natural killer (NK) T cells, γδ T cells, or innate lymphoid cells (ILCs) in the lungs of *Legionella*-infected mice (fig. S3C). There was no significant difference in bacterial load (CFU) at day 7 in the lungs of *Icos*$^{-/-}$ or *Il-23p19*$^{-/-}$ mice compared with WT mice, although *Il-23p19*$^{-/-}$ mice had slightly higher load by 10 days p.i. (fig. S3D).

The expression of RORγt and T-bet appears to be mutually exclusive in naïve MAIT cells (*17*, *31*), but intranasal infection with *S.* Typhimurium induced expression of T-bet, resulting in a large proportion of MAIT cells expressing both transcription factors (*19*). Given the requirement for IL-23 in the expansion of MAIT cells (Fig. 4, A and B) and its known role in driving differentiation of naïve conventional T cells to T_H17 subsets (*46*–*54*), we determined how IL-23 was involved in altering transcription factor expression in MAIT cells after infection. Upon infection with *S.* Typhimurium BRD509, the proportion of MAIT cells expressing T-bet was lower in *Il-12p35*$^{-/-}$ and *Il-12p40*$^{-/-}$ mice compared with WT mice (Fig. 4C). In *Il-23p19*$^{-/-}$ mice, the proportion of T-bet$^+$ RORγt$^-$ MAIT cells was increased during bacterial infection, whereas in WT mice, most MAIT cells coexpressed T-bet and RORγt (Fig. 4C). In addition, we observed similar changes in the transcriptional factor expression by the MAIT cell population in *Il-23p19*$^{-/-}$ mice infected with *L. longbeachae* NSW150 (fig. S3E).

In *S.* Typhimurium–infected WT mice, ~50% of MAIT cells and 12% of non-MAIT αβ T cells isolated from the lungs produced IL-17A, as detected by intracellular cytokine staining 7 days p.i. (Fig. 4, D and E). *Il-6*$^{-/-}$ mice showed a significant decrease in non-MAIT T cells, but not MAIT cells, producing IL-17A (Fig. 4, D and E). In contrast, *Il-23p19*$^{-/-}$ mice had lower numbers of MAIT cells (Fig. 4B), and only 5 to 15% of these MAIT cells produced IL-17A, consistent with a lower expression of RORγt (Fig. 4, C and E). Non-MAIT T cells required both IL-6 and IL-23 for optimal IL-17 production, consistent with previous reports (*55*) (Fig. 4, D and E). Overall, these data suggest that IL-23, but not IL-6

Downloaded from http://immunology.sciencemag.org/ by guest on January 25, 2021

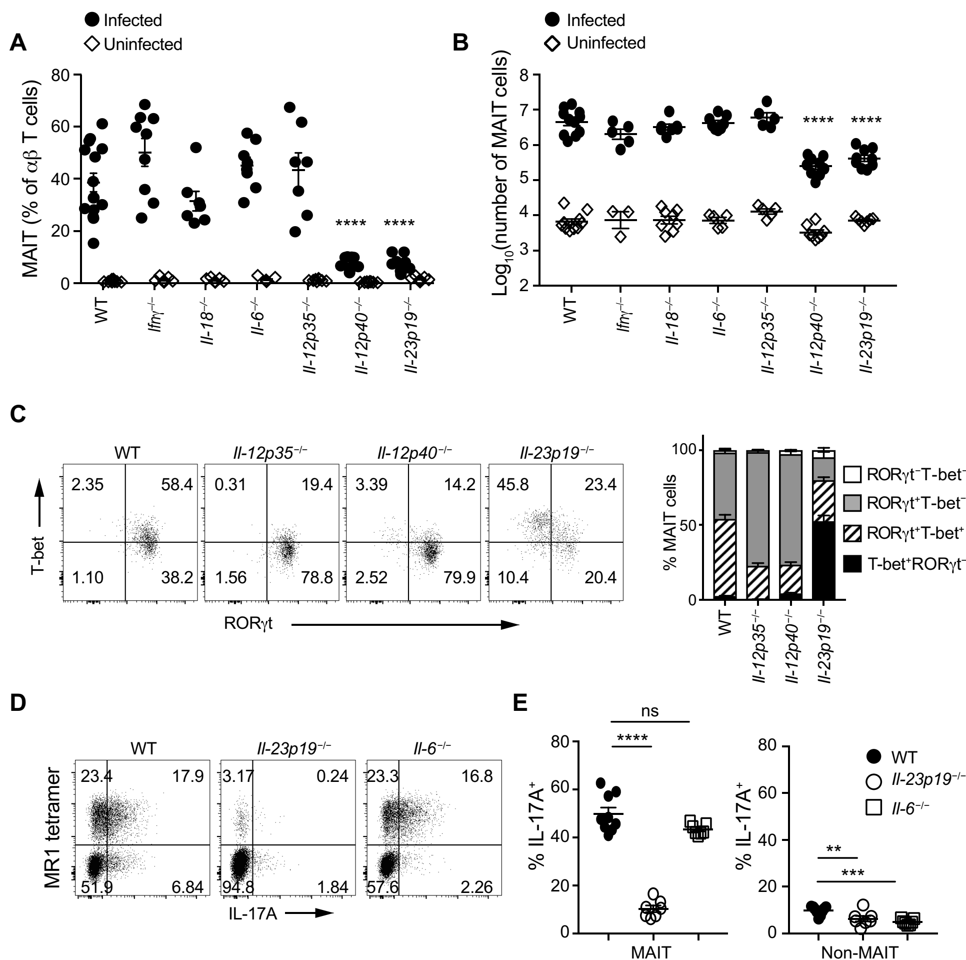

Fig. 4. IL-23 is required for MAIT cell accumulation and activation during pulmonary infection with *S.* Typhimurium. (**A**) Percentage and (**B**) absolute numbers of MAIT cells isolated from the lungs of gene KO mice lacking indicated cytokines after intranasal infection with 10^6 *S.* Typhimurium BRD509 (day 7 p.i.). Data show individual mice and means ± SEM of 3 to 15 mice per group. ****$P < 0.0001$ (all uninfected mice non-significant), one-way ANOVA with Dunnett's multiple comparisons for infected mice. (**C**) Representative flow cytometry plots and stacked plots showing intracellular staining of T-bet and RORγt in MAIT cells from *S.* Typhimurium–infected *Il-12p35*$^{-/-}$, *Il-12p40*$^{-/-}$, *Il-23p19*$^{-/-}$, and WT (C57BL/6) mice (day 7 p.i.). (**D**) Flow cytometry plots and (**E**) percentages of pulmonary TCRβ$^+$ lymphocytes (non-MAIT T cells and MAIT cells) producing IL-17A by intracellular staining, directly ex vivo from the lungs of WT (C57BL/6), *Il-23p19*$^{-/-}$, and *Il-6*$^{-/-}$ mice infected with 10^6 *S.* Typhimurium BRD509 (day 7 p.i.). Data show means ± SEM and individual mice for eight to nine mice per group. **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$, one-way ANOVA with Dunnett's multiple comparisons. See also fig. S3.

or other cytokines tested, is essential for MAIT cell accumulation in the lungs and for maintaining a MAIT-17 profile after bacterial lung infection.

Exogenous IL-23 restores MAIT cell capacity to proliferate in *Il-23p19*$^{-/-}$ mice

To confirm the requirement for IL-23 in driving the accumulation of MAIT cells in the lungs after infection, we investigated whether restoring IL-23 would rescue MAIT cell accumulation after infection in *Il-23p19*$^{-/-}$ mice. To do this, we administered a plasmid construct coding for the p19 and p40 subunits of IL-23 fused to an immunoglobulin (Ig) Fc region [IL-23–Ig (*56*)] to *Il-23p19*$^{-/-}$ mice via hydrodynamic injection 1 day before intranasal infection with *S.* Typhimurium BRD509 and then assessed MAIT cell accumulation in the lungs at day 7 p.i. IL-23 was readily detectable in bronchoalveolar lavage fluid (BALF) of mice receiving IL-23–Ig plasmid by 8 hours, and at 24 hours, after hydrodynamic injection (fig. S4A), consistent with the expected kinetics of protein expression by this method (*57*). In *Il-23p19*$^{-/-}$ mice administered with IL-23–Ig, but not a control plasmid, accumulation of MAIT cells comparable with that found in WT mice at day 7 p.i. was observed (Fig. 5, A and B). The transcription factor profile of these accumulating cells shifted toward coexpression of RORγt and T-bet (MAIT17/MAIT-1 phenotype) and resembled that observed in WT mice (Fig. 5C). These data demonstrate that MAIT cells in *Il-23p19*$^{-/-}$ mice are capable of proliferating and maintaining expression of RORγt, and this depends on the presence of IL-23. The administration of IL-23–Ig plasmid DNA, but not control plasmid, also increased the expression of ICOS on MAIT cells relative to untreated control mice (Fig. 5D).

MAIT cells express high levels of IL-23 receptor and respond to IL-23 in a direct manner

To determine whether IL-23 also affected MAIT cells directly during bacterial infection, we examined the expression of IL-23 receptor (IL-23R) using the *Il-23r*$^{gfp/+}$ heterozygous mice [reporter mice in which green fluorescent protein (GFP) expression is linked to IL-23 expression] (*58*). Flow cytometric detection of GFP in lung αβ T cells showed that >85% of MAIT cells expressed IL-23R in naïve reporter mice (Fig. 6A). During acute bacterial infection (day 7), MAIT cells that accumulated in the lungs down-regulated expression of IL-23R, with expression restored to ~74% in mice long-term after infection (Fig. 6A). Next, we adoptively transferred WT MAIT cells (sourced from the lungs of long-term *S.* Typhimurium–infected mice) into either *Il-23r*$^{-/-}$ mice, as a model in which only the transferred MAIT cells could respond to IL-23, or *Il-23p19*$^{-/-}$ mice, and infected these mice with *S.* Typhimurium (Fig. 6B). After infection, adoptively transferred WT MAIT cells accumulated to a much greater extent (~143-fold) in the lungs of *Il-23r*$^{-/-}$ mice compared with those in *Il-23p19*$^{-/-}$ mice (~7-fold) and compared with the expansion of endogenous MAIT cells present in *Il-23r*$^{-/-}$ mice (~15-fold) (Fig. 6, B and C, and fig. S5). Endogenous MAIT cells in either *Il-23p19*$^{-/-}$ or *Il-23r*$^{-/-}$ mice expanded similarly (23- and 15-fold, respectively). This finding suggests that both the availability of IL-23 and the expression of IL-23R on MAIT cells are essential for optimal MAIT cell expansion during bacterial infection,

Downloaded from http://immunology.sciencemag.org/ by guest on January 25, 2021

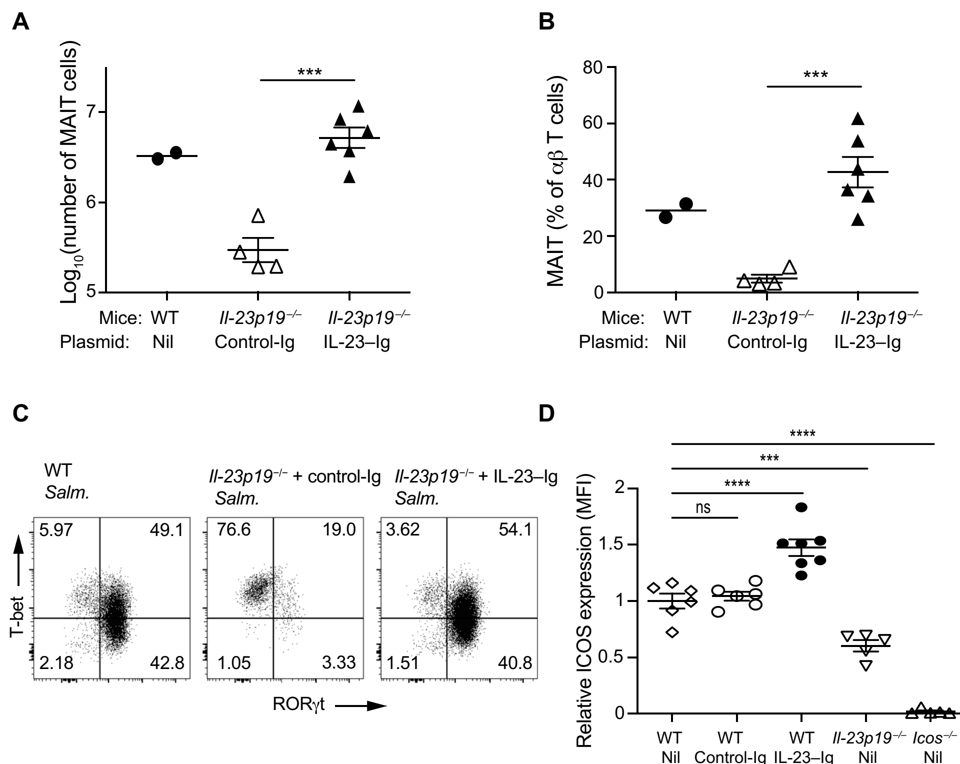

Fig. 5. Infused IL-23–Ig plasmid restores MAIT cell accumulation in *Il-23p19*$^{-/-}$ mice after infection. (**A**) Absolute numbers and (**B**) MAIT cell percentage of T cells isolated from the lungs of WT and *Il-23p19*$^{-/-}$ mice intranasally infected with 10^6 *S.* Typhimurium BRD509 (day 7 p.i.). *Il-23p19*$^{-/-}$ mice were treated with 10 μg of plasmid encoding recombinant IL-23–Ig or control Ig by hydrodynamic injection 1 day before infection. Data show means ± SEM and individual mice. ***$P < 0.001$, unpaired two-tailed Student's *t* test. (**C**) Expression of T-bet and RORγt in MAIT cells from lungs of mice described above. Plots show gated MAIT cells from one representative mouse per group. (**D**) Relative ICOS expression (compared with WT C57BL/6 mice) on pulmonary MAIT cells from untreated (nil) WT, *Il-23p19*$^{-/-}$ or *Icos*$^{-/-}$ mice, or WT mice treated with 10 μg of IL-23–Ig or control-Ig plasmid for 24 hours. Data show means ± SEM and individual mice. ***$P < 0.001$, ****$P < 0.0001$, one-way ANOVA with Dunnett's multiple comparisons test. See also fig. S4.

expression, an indicator of cytokine production, resembled that in mice infected with *S.* Typhimurium (Fig. 3C). Thus, IL-23 provides a sufficient costimulatory signal to trigger MAIT cell expansion and activation in vivo and skews MAIT cells toward a MAIT-17/1 phenotype.

We next sought to determine whether these findings could be recapitulated with human MAIT cells. To this end, we stimulated healthy donor peripheral blood mononuclear cells (PBMCs) in vitro with 5-OP-RU in the presence of IL-2, with and without IL-23, for 13 days to allow time for the cells to proliferate to high enough numbers and consolidate their cytokine profiles for analysis. Cells were then stained intracellularly to detect production of IL-17 and IFN-γ. In the absence of IL-23, 5-OP-RU induced a strong IFN-γ response, whereas when 5-OP-RU was combined with IL-23, MAIT cells increased IL-17 production and the percentage of IFN-γ–producing MAIT cells was reduced (fig. S6, A and B). IL-23 alone induced minimal cytokine production. Thus, similar to mouse MAIT cells, the presence of IL-23 during Ag stimulation drives human MAIT cells toward a MAIT-17–type response.

although other cytokines may drive a milder response. Together, our data demonstrate that MAIT cells express high levels of IL-23R and respond to IL-23 directly, resulting in their expansion at the site of infection.

IL-23 drives MAIT cell expansion in an MR1-Ag–dependent manner

We next tested whether IL-23 would be a sufficient costimulatory signal for MAIT cell accumulation and activation in vivo in response to synthetic Ag. To address this question, we infused IL-23–Ig plasmid DNA hydrodynamically into WT mice 1 day before intranasal administration of synthetic 5-OP-RU. We detected a significant expansion of MAIT cells in the lungs of mice treated with IL-23–Ig, but not with a control plasmid, 7 days after initial 5-OP-RU inoculation (Fig. 7A and fig. S4B). This enrichment of MAIT cells occurred in a dose (of 5-OP-RU)–dependent manner (Fig. 7A and fig. S4B), although there was a small but significant effect on the absolute number of MAIT cells with IL-23–Ig alone (Fig. 7A). None of the inoculations had any obvious effect on the absolute number of non-MAIT T cells in the lungs (Fig. 7B). In addition, either IL-23–Ig or 5-OP-RU alone induced some up-regulation of T-bet in MAIT cells, but this effect was enhanced when IL-23–Ig was combined with 5-OP-RU (Fig. 7C) such that the profile of transcription factor

IL-23 plus 5-OP-RU–primed MAIT cells can enhance control of *L. longbeachae*

To determine whether MAIT cells primed with 5-OP-RU in combination with IL-23 could augment protective immunity to bacterial infection, we first vaccinated mice with IL-23–Ig via hydrodynamic injection (once, day 0) via tail vein combined with intranasal administration of synthetic 5-OP-RU (four times, days 1 to 4) (MAIT cell vaccination). After 4 to 5 weeks, these mice, as well as control mice receiving IL-23–Ig alone or control-Ig DNA together with 5-OP-RU, were challenged intranasally with 10^4 CFU *L. longbeachae*, and the bacterial load in the lungs was measured by CFU assay at days 5 and 7 p.i. (Fig. 7D). In control mice, the bacterial load decreased significantly between days 5 and 7 p.i., indicating bacterial clearance, consistent with our previous data in WT C57BL/6 mice (*20*). Bacterial loads were significantly reduced in mice that had received Ag-specific MAIT cell vaccination with 5-OP-RU in combination with IL-23–Ig, compared with control mice (untreated, treated with IL-23–Ig alone, or treated with control-Ig plus 5-OP-RU) (Fig. 7E). The difference was most evident 5 days p.i., when a 50-fold reduction in bacterial load was observed in vaccinated (IL-23–Ig + 5-OP-RU) mice relative to controls.

DISCUSSION

Our findings demonstrate that MAIT cell activation in vivo not only is dependent on MR1 and antigen but also requires costimulatory signals and that ultimate expansion of MAIT cells is augmented

Downloaded from http://immunology.sciencemag.org/ by guest on January 25, 2021

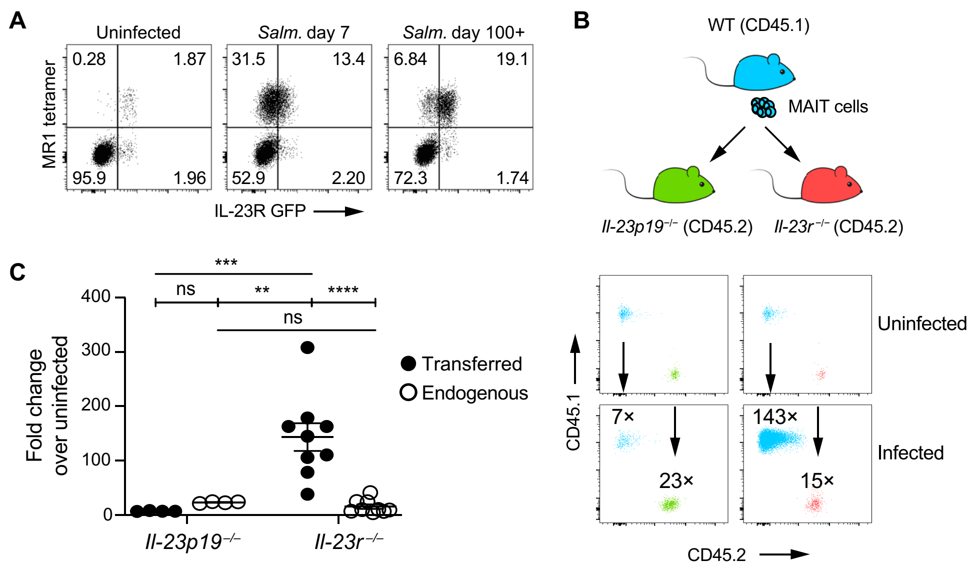

Fig. 6. MAIT cells express IL-23R and respond directly to IL-23. (**A**) GFP expression (indicating IL-23R expression) in TCRβ$^+$ cells isolated from the lungs of *IL-23r*$^{gfp/+}$ uninfected mice or after intranasal infection with 10^6 *S.* Typhimurium BRD509 (day 7 or day 100 p.i.). (**B**) Schematic of MAIT cell transfer and tracking using CD45 congenic markers. Plots show MAIT cells (CD45$^+$TCRβ$^+$MR1–5-OP-RU–Tetramer$^+$) isolated from the lungs of recipient mice color-coded to match the donor/recipient cells. Fold increase of MAIT cell number after infection is shown. (**C**) Total MAIT cells (CD45.2$^+$ endogenous and CD45.1$^+$ adoptively transferred) isolated from the lungs of *Il-23p19*$^{-/-}$ or *Il-23r*$^{-/-}$ mice that received 10^5 MAIT cells (sorted from CD45.1 congenically labeled mice primed for 7 days with *S.* Typhimurium BRD509) and, 2 weeks later, were intranasally infected with 10^6 *S.* Typhimurium BRD509. Data show means ± SEM and individual mice fold change of normalized data at day 7 p.i. $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$; ns, $P > 0.05$, one-way ANOVA with Tukey's multiple comparisons test. See also fig. S5.

by the inflammatory cytokine IL-23. Although MR1 is ubiquitously expressed at low levels (*11*–*13*) and both hematopoietic and epithelial cell lines can activate MAIT cells in vitro (*29*, *59*), the source of cells responsible for presenting Ag to MAIT cells in vivo have not been described. Using various combinations of WT ↔ *Mr1*$^{-/-}$ BM chimeras, we show here that both BM- and non–BM-derived cells are capable of driving MAIT cell activation, but the predominant APC type is determined by the nature of the bacterial infection. *L. longbeachae* does not actively invade cells but replicates in alveolar macrophages after phagocytosis (*60*). Consequently, *L. longbeachae* is confined to BM-derived lung macrophages that provide the necessary signals for MAIT cell activation. In contrast, for *S.* Typhimurium, which actively infects many cell types, including epithelial cells (*61*), non–BM-derived cells were more influential than BM-derived APC for the activation of MAIT cells. The low capacity of BM-derived APC to drive activation of MAIT cells in this context may be due to inhibitory mechanisms evolved by *S.* Typhimurium (*62*–*64*).

In addition to Ag, T cell activation requires a secondary "costimulatory" signal, and we found that ICOS, but not CD80 or CD86, exerted a significant impact on MAIT cell activation in vivo. This finding is consistent with the high constitutive level of surface expression of ICOS on MAIT cells compared with non-MAIT T cells in naïve mice. ICOS costimulation is involved in a number of other processes during adaptive immune responses, including the formation of T follicular helper cells and enhancing or dampening T_H1 and T_H2 inflammatory responses, depending on the pathogen (*65*, *66*), as well as costimulation of cytokine production by memory CD4$^+$ T cells (*67*). The significantly lower accumulation of MAIT cells in *Icos*$^{-/-}$ mice after either *Salmonella* or *Legionella* infection suggests that ICOS signaling promotes MAIT cell proliferation. This finding contrasted with non-MAIT T cell and ILC numbers that were unaffected by ICOS deficiency. ICOS-L can be expressed on both BM-derived APCs and non–BM-derived cells (*67*–*71*). The greater dependence of MAIT cell expansion in *Icos*$^{-/-}$ mice during infection with *Salmonella* compared with *Legionella* may reflect the greater importance of ICOS as a costimulatory signal on epithelial versus BM-derived cells.

In contrast to conventional naïve T cells (*72*) and CD1d-restricted invariant NK T cells (*73*) where CD28-CD80/CD86 interactions are critical to activation, CD80/86-CD28 signaling did not appear to be important for MAIT cell activation, perhaps consistent with the description of MAIT cells as "effector memory" cells (*14*–*17*). Other costimulatory molecules such as CD137 and CD154 are expressed at low levels on naïve MAIT cells, although a proportion of human MAIT cells up-regulated CD137 (4-1BB) when cultured with *Mycobacterium tuberculosis* lysate (*74*). Thus, our study does not rule out a role for CD137 during infection.

Most of the MAIT cells in naïve mice express RORγt, and the expression of RORγt and T-bet can be mutually exclusive (*17*), but after infection, most of the MAIT cells express both transcription factors (*19*). Double-positive RORγt and T-bet MAIT cells were also observed in *Icos*$^{-/-}$ mice but with an increase in proportion of MAIT-1 (T-bet$^+$RORγt$^-$, T_H1-like) cells, suggesting a role for ICOS in maintaining a MAIT-17 phenotype during infection, as for conventional CD4$^+$ and CD8$^+$ T cells (*66*, *75*). An ICOS agonist confers more potent T cell function through an increase in inflammatory cytokines and IL-23R expression (*66*, *75*).

IL-23 is required for the functional differentiation of IL-17–producing T_H17 cells, stabilizing their phenotype through the transcription factor signal transducer and activator of transcription 3 (STAT3) pathway (*76*, *77*). Our findings indicate that IL-23 is also required for MAIT cell proliferation and differentiation, the addition of exogenous IL-23 fully reconstituting the MAIT cell proliferation and phenotype in IL-23–deficient mice. STAT3 loss-of-function mutations result in deficiency of human MAIT cells and other cells (*78*). Although not directly tested here, we speculate that IL-23 also acts through STAT3 in driving the differentiation of MAIT-17 cells.

MAIT cell transfer experiments also demonstrated that IL-23 acts directly on MAIT cells consistent with the high expression level of IL-23R on MAIT cells (*14*, *79*) and with previous studies, where the cytokine appeared critical for prolonged survival of T_H17 cells, consolidated the T_H1 phenotype, and conferred full T_H17 pathogenic function potential (*39*, *80*, *81*). Consistent with findings in other T cell subsets (*82*), IL-23, from plasmid DNA, enhanced ICOS expression on MAIT cells, suggesting an indirect effect, via augmented ICOS expression, on MAIT cell activation. In contrast, unlike CD4$^+$ T_H17 cells (*39*, *83*), MAIT cell development was not dependent on

Downloaded from http://immunology.sciencemag.org/ by guest on January 25, 2021

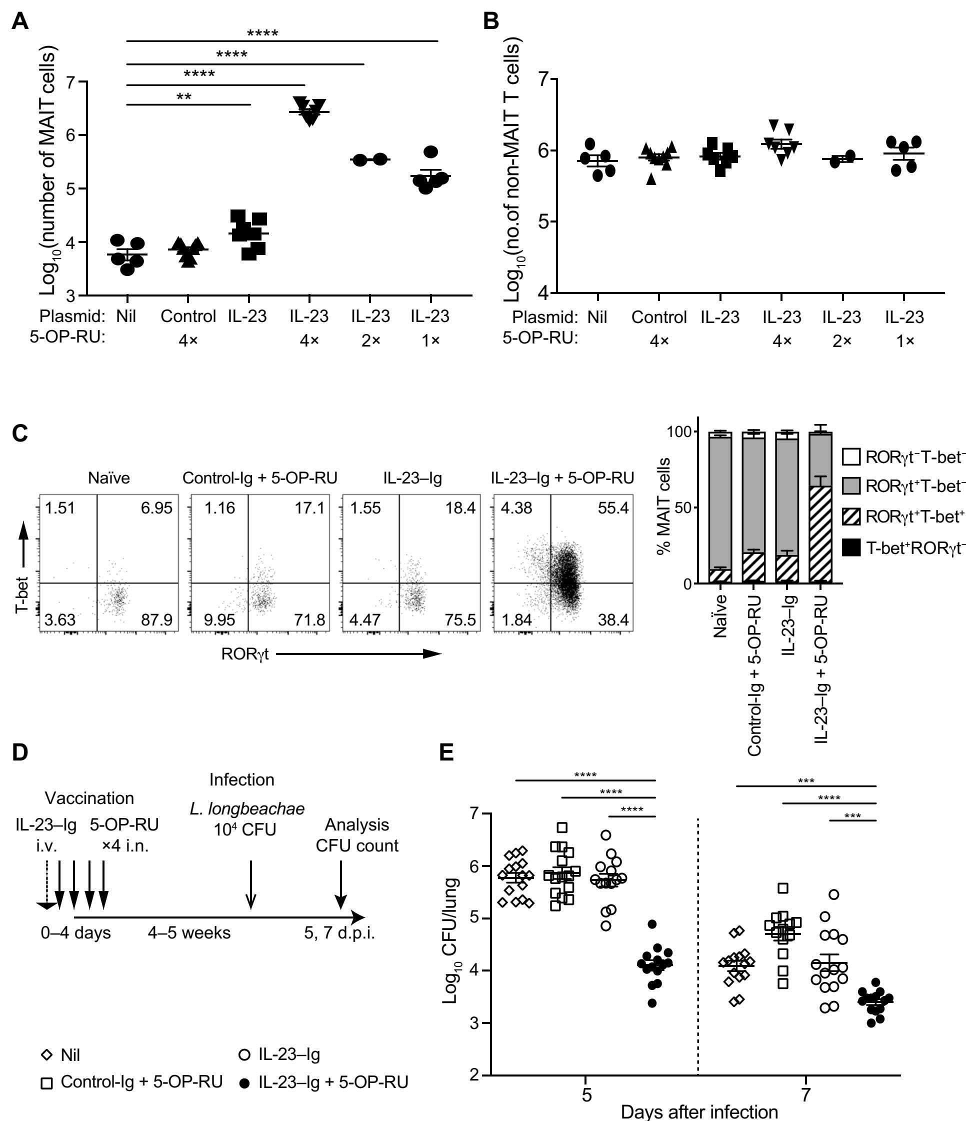

Fig. 7. IL-23 plus synthetic 5-OP-RU antigen is sufficient to induce MAIT cell activation and accumulation in vivo and increase protection against infectious challenge. (**A**) Absolute numbers of MAIT cells and (**B**) absolute number of non-MAIT TCRβ$^+$ cells isolated from the lungs on day 8 of naïve WT (C57BL/6) mice or WT mice treated with 10 μg of plasmid encoding recombinant IL-23–Ig or control plasmid by hydrodynamic injection (day 0) and inoculated intranasally with one to four doses (as indicated) of 5-OP-RU on days 0, 1, 2, and 4. Data show means ± SEM and individual mice. (**C**) Expression of T-bet and RORγt in MAIT cells from the lungs of mice described in (A). Plots show data from one representative mouse from each group. (**D**) Experimental scheme for vaccination experiments shown in (E). d.p.i., days post infection. (**E**) Bacterial CFU counts of *Legionella* from the lungs at indicated time points after intranasal infection with 10^4 CFU of mice previously untreated (open diamonds) or primed with IL-23–Ig (hydrodynamic injection) alone (open squares) or IL-23–Ig or control-Ig with 5-OP-RU (four doses intranasally) (closed and open circles, respectively). Data show means ± SEM and individual mice. **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$, one-way ANOVA with Dunnett's multiple comparisons test. See also fig. S4.

IL-6, demonstrating a key difference in the regulation of MAIT cell activation compared with conventional T_H17 cells. MAIT cell expansion appeared to be independent of bacterial antigen load because CFU counts were similar at the peak of infection in WT, *Icos*$^{-/-}$, and *Il-23*$^{-/-}$ mice. Moreover, neither IL-12 nor IL-18 was required for MAIT cell expansion after infection, consistent with the fact that in vitro observations do not always reflect in vivo responses (*84*).

However, MAIT cells expanded, albeit at a much lower level, in IL-23–deficient mice, suggesting that there may be other activation pathways for MAIT cells and that IL-23 may play a synergistic role in the maximal activation of MAIT cells. In addition to their role in clearing bacterial infection, demonstrated by our previous studies (*20*) and by others for different bacteria (*85*–*87*), it has also been proposed that MAIT cells act to protect barrier integrity (*88*) and have a tissue repair phenotype (*89*). At this stage, the mechanisms linking MAIT cell expansion and pathogen clearance are unclear.

Vaccination of mice with 5-OP-RU in combination with IL-23 augmented bacterial clearance at early time points (most evident at day 5 p.i.), consistent with the MAIT cell vaccination effect shown in our previous study where we primed MAIT cells with 5-OP-RU and CpG (*20*). In contrast, the effect of MAIT cell deficiency on bacterial loads was most evident at day 10 in a primary *L. longbeachae* infection (*20*). Thus, priming of MAIT cells is consistent with the concept that MAIT cells can form a population of cells with memory-like recall properties. Understanding the signals that drive MAIT cells during infection is fundamental to strategies that might enhance protection against subsequent pulmonary infections. Accordingly, our findings not only verify the role of MAIT cells in protective immunity but also suggest that this might be boosted for specific benefit under some circumstances. Because MAIT cells can produce both IFN-γ and IL-17, both of which have been shown to be important for clearance of *Legionella* infections (*90*–*92*), it will be important to determine the mechanisms by which MAIT cells contribute to bacterial clearance in this setting.

Our data demonstrate that MAIT cell activation requirements are broadly similar but distinct from those of other T cells. Ag presented by MR1 constitutes an indispensable signal 1, and both BM- and non–BM-derived cells could act as APCs, depending on the pathogen. Among costimulating receptors (signal 2), ICOS appears critical for optimal MAIT cell activation. IL-23 acts directly on MAIT cells and may also act indirectly through up-regulating ICOS expression, promoting cell proliferation, and skewing MAIT cells to a MAIT-17/1 profile. Combined vaccination with IL-23–Ig and 5-OP-RU was able to activate MAIT cells and confer enhanced protection against bacterial infection, demonstrating the therapeutic potential

Downloaded from http://immunology.sciencemag.org/ by guest on January 25, 2021

of modulating MAIT cells by harnessing the molecular signals that drive this innate-like T cell subset.

MATERIALS AND METHODS

Study design

The goal of the study was to determine the requirements for MAIT cell activation in vivo, particularly in the context of bacterial infection. Flow cytometry was used to enumerate and characterize MAIT cells, and the bacterial load was measured by CFU counts, from the lungs of WT C57BL/6 mice and in different gene-deficient mice. Analysis was conducted after infection with *Legionella* or *Salmonella* and after priming with antigen and cytokines delivered as plasmid DNA and also in human PBMC MAIT cells after in vitro culture under different conditions. BM chimeras and adoptive transfers of congenically marked cells were used to validate findings and to understand which cell types were important for expression of MR1 and cytokine receptors. For mouse studies, sample sizes were chosen according to the power of the statistical test of each experiment that is depicted in the figures, and the number of independent experiments is listed in the figure legends. Mice were euthanized either at experimental end points or at humane end points according to institute ethics approvals. The investigators were blinded when performing immunizations and infections but not blinded for analysis. Different groups of mice were age- and sex-matched. Both male and female mice were used.

Mice and infections

Mice were bred and housed in the Biological Research Facility of the Peter Doherty Institute for Infection and Immunity (Melbourne, Victoria, Australia). *Mr1*$^{-/-}$ mice were generated by breeding Vα19*i*C$\alpha^{-/-}$*Mr1*$^{-/-}$ mice (*93*) (from Susan Gilfillan, Washington University, St. Louis School of Medicine, St. Louis, MO) with C57BL/6 mice and intercrossing of F1 mice. The genotype was determined by tail DNA polymerase chain reaction at the *Mr1* locus as previously described (*19*). IL-23R GFP reporter mice (*Il-23r*$^{+/gfp}$) were F1 mice from breeding of C57BL/6 and homozygous *Il-23r*$^{gfp/gfp}$ mice. To generate BM chimeras, we lethally irradiated (2 × 5.5 gray, 3 hours apart) recipient mice to deplete BM-derived cells. Donor BM cells (10^7) were injected to the recipient mice via the tail vein. The mice were then rested for 8 weeks before infection to allow full reconstitution of their BM-derived cells. Mice aged 6 to 12 weeks (except BM chimeras) were used in experiments, after approval by the University of Melbourne Animal Ethics Committee.

Compounds, immunogens, and tetramers

5-OP-RU was prepared as described previously (*94*) and diluted to the desired concentration in phosphate-buffered saline (PBS) just before use. Murine and human MR1 and β2-microglobulin genes were expressed in *Escherichia coli* inclusion bodies, refolded, and purified as described previously (*2*, *95*). MR1–5-OP-RU and MR1–6-FP tetramers were generated as described previously (*1*). CD1d-αGalCer tetramer–phycoerythrin (PE) was provided by D. Godfrey (University of Melbourne).

Bacterial strains

Cultures of *L. longbeachae* NSW150 were grown at 37°C in buffered yeast extract (BYE) broth supplemented with streptomycin (30 to 50 μg/ml) for ~16 hours with shaking at 180 rpm to reach log phase [optical density at 600 nm (OD_{600}), 0.2 to 0.6]. For the infecting inoculum, bacteria were reinoculated in prewarmed medium for a further 2- to 4-hour culture (OD_{600}, 0.2 to 0.6), and with the estimation that 1 $OD_{600} = 5 \times 10^8$/ml, sufficient bacteria were washed and diluted in PBS with 2% BYE for intranasal delivery to mice. A sample of inoculum was plated onto buffered charcoal yeast extract (BCYE) agar plates with streptomycin for verification of bacterial inoculum dose by counting CFU.

For infection of adoptive transfer donor mice with *S.* Typhimurium BRD509, cultures were prepared as previously described (*19*). SL1344 and SL1344Δ*invA* (χ4370; originally provided by R. Curtiss III, Department of Biology, Washington University, St. Louis, MO) were grown in LB or on Luria agar plates containing appropriate antibiotics. Inoculum preparation and intranasal infection of mice were performed as previously described (*19*).

For infection of cultured cells with *S.* Typhimurium or mutants, human and murine cells (10^6 per well) of either epithelial [human: BSB-II; mouse: LET1 (*96*)] or macrophage (human: THP-1; mouse: BMDM) origin were cultured in 24-well plates in triplicate, washed once with Dulbecco's modified Eagle's medium (DMEM) without antibiotics, resuspended in 10% fetal bovine serum (FBS) DMEM, and cocultured with bacteria prepared at log phase at a defined multiplicity of infection (MOI) for 1 hour. The cells were then washed three times with DMEM containing 10% FBS and antibiotics, resuspended in 0.5 ml of 10% FBS DMEM containing gentamicin (100 μg/ml), and cultured for 1.5 hours at 37°C to kill extracellular bacteria. Cells were then harve sted, washed twice with PBS, lysed with 0.2 ml of 0.1% digitonin, spread on Luria agar plates containing appropriate antibiotics, and incubated at 37°C overnight for bacterial counts.

Intranasal infection

Intranasal inoculation with *S.* Typhimurium, *L. longbeachae*, or antigens (76 pmol 5-OP-RU) in 50 μl per nares was performed on isoflurane-anesthetized mice. Mice were euthanized by CO_2 asphyxia at different time points after infection, and the lungs were perfused with 10 ml of cold RPMI 1640 via heart injection before being taken for further processing.

To prepare single-cell suspensions, we finely chopped the lungs with a scalpel blade and digested them with collagenase III (3 mg/ml; Worthington, Lakewood, NJ), deoxyribonuclease (5 μg/ml), and 2% FBS in RPMI 1640 for 90 min at 37°C with gentle shaking. Cells were then filtered (70 μm) and washed with PBS/2% FBS. Red blood cells were lysed with hypotonic tris-buffered ammonium chloride solution for 5 min at 37°C. About 1.5×10^6 cells were stained with MR1 tetramers and antibodies, washed, and filtered (40 μm) before flow cytometric analysis.

Determination of bacterial counts in infected lungs

Bacterial load in the lungs was determined by counting CFU obtained from plating homogenized lungs in duplicate from infected mice (×5 per group) on BCYE agar containing streptomycin (30 μg/ml). Colonies were counted after 4 days at 37°C under aerobic conditions.

Adoptive transfer

Because MAIT cell frequencies are low in naïve C57BL/6 mice, before adoptive transfer experiments, MAIT cell populations were expanded by intranasal infection with 10^6 CFU *S.* Typhimurium BRD509 in

Downloaded from http://immunology.sciencemag.org/ by guest on January 25, 2021

50 μl of PBS for 7 days as previously described (*19*). After 7 days, mice were euthanized, single-cell suspensions were prepared, and live TCRβ$^+$CD45$^+$MR1–5-OP-RU tetramer$^+$ cells were sorted using a BD FACSAria III. A total of 10^5 MAIT cells were injected into recipient mice through the lateral tail vein. Mice were rested for 2 weeks after transfer to allow the MAIT cell population to settle to homeostasis before subsequent infectious challenge.

Jurkat.MAIT reporter cell activation assays

MAIT cell reporter activation assays were performed essentially as previously reported (*2*, *37*). Jurkat cells overexpressing the MAIT TCR clone AF-7 (Jurkat.MAIT) were tested for activation by coincubation with compounds and C1R cells overexpressing MR1 (CIR.MR1) for 16 hours. Cells were subsequently stained with PE-Cy7–conjugated anti-CD3 (1:300; UCHT1, eBioscience) and APC-conjugated anti-CD69 (1:25; BD Biosciences) before analysis using a FACS Canto II (BD Biosciences) flow cytometer. Activation of Jurkat.MAIT was measured by an increase in surface CD69 expression.

In vitro human PBMC assays

Human PBMCs from healthy donors were obtained from the Australian Red Cross Blood Service (ARCBS) after approval from the University of Melbourne Human Research Ethics Committee (1853263.3). In four independent experiments, PBMCs were stimulated with 0.1 μM 5-OP-RU in the absence or presence of recombinant human IL-23 (20 ng/ml) for 10 to 12 days [IL-2 (10 units/ml) was added at day 5] before restimulation with C1R-MR1 APCs in the presence or absence of 5-OP-RU for 5 hours. Brefeldin A (BD Biosciences) was added during the last 4 hours. Viable cells (defined using Aqua LIVE/DEAD Dye, Thermo Fisher Scientific) were then stained with anti-CD3 (PE-CF594), anti-CD8α (APC), anti-CD4 (APC-Cy7), and anti-Vα7.2 (BV785) monoclonal antibodies (mAbs), and MR1–5-OP-RU–streptavidin-PE tetramer, after which cells were permeabilized and fixed (eBioscience) before intracellular staining with anti–IL-17A (PE-Cy7) and anti–IFN-γ (AF700) mAbs. Stained cells were analyzed on an LSRFortessa flow cytometer (BD Biosciences).

Antibodies and flow cytometry

Antibodies against murine CD3 (145-2C11, PE-Cy7), CD4 (GK1.5, APC-Cy7), CD19 (1D3, PerCP-Cy5.5), CD137 [1AH2, fluorescein isothiocyanate (FITC)], CD45.2 (104, FITC), IFN-γ (XMG1.2, PE-Cy7), TCRβ (H57-597, APC, or PE), and IL-17A (TC11-18H10, PE) were purchased from BD (Franklin Lakes, NJ). Antibodies against CD8α (53-6.7, PE), CD45.1 (A20, PE), CD27 (LG.7F9, APC), RORγt (B2D, APC), and T-bet (4B10, PE-Cy7) were purchased from eBioscience (San Diego, CA). Abs against CD45.1 (A20, FITC), CD28 (E18, FITC), CD154 (MR1, PE), ICOS (7E.17G9, PE), CD86 (PO3, PE), TCRγδ (GL3, APC), CD127 (A7R34, APC), CD3 (17A2, PerCPCy5.5), and Lineage cocktail (FITC) were purchased from BioLegend (San Diego, CA). To block nonspecific staining, we incubated cells with MR1–6-FP tetramer and anti-Fc receptor (2.4G2) for 15 min at room temperature and then incubated them at room temperature with Ab/tetramer cocktails in PBS/2% FBS. 7-Aminoactinomycin D (Sigma-Aldrich) was added during antibody staining.

Antibodies against human CD3 (UCHT1, PE–Alexa Fluor 594), TCR-Vα7.2 (3C10, APC), CD161 (HP-3G10, PE-Cy7), TNF-α (MAb11, Pacific Blue), and viability dye (Zombie Yellow) were purchased from BioLegend. Antibodies against IFN-γ (25725.11, FITC) and CD69 (FN50, PE) were purchased from BD, and anti-CD3 (UCHT1, APC) was purchased from eBioscience.

Cells were fixed with 1% paraformaldehyde before analysis on LSRII, LSRFortessa, or Canto II (BD Biosciences) flow cytometers. For intracellular cytokine staining, Golgi plug (BD Biosciences) was used during all processing steps. Cells stimulated with PMA (phorbol 12-myristate 13-acetate)/ionomycin (20 ng/ml and 1 μg/ml, respectively) for 3 hours at 37°C were included as positive controls. Fixable Viability Dye (eBioscience) was added for 30 min at 4°C before surface staining. Surface staining was performed at room temperature, and cells were stained for intracellular cytokines using the BD Fixation/Permeabilization Kit (BD, Franklin Lakes, NJ) or transcription factors using the transcription buffer staining set (eBioscience) according to the manufacturers' instructions. Flow cytometric data analysis was performed with FlowJo 10 software. Gating for MAIT cells was performed as previously described (*20*).

Constructs, hydrodynamic injection, and MAIT antigen delivery

IL-23–Ig plasmid (pEF-BOS–IL-23–IgG3) and control-Ig (pEF-BOS-IgG3) constructs were provided by Burkhard Becher, Switzerland. IL-23 expression from the plasmid has been previously characterized (*56*, *97*). Hydrodynamic injection was performed as described elsewhere (*98*), by placing prewarmed mice in a prewarmed conical restraining device. Ten micrograms of a plasmid vector encoding IL-23–Ig or control vector was injected in 1.6 to 1.8 ml of TransIT-EE Hydrodynamic Delivery Solution (MIR 5340, Mirus Bio LLC) over a period of 10 s (*99*). MAIT antigen (5-OP-RU; 50 μl, 1 μM) was delivered intranasally four times (on days 0, 1, 2, and 4), twice (on days 0 and 1), or once (on day 0) after hydrodynamic injection. Mice were then euthanized on day 7 for examination of MAIT cell number and function or left for a month for vaccinated MAIT cells to form memory before challenge with *L. longbeachae* as described previously (*20*).

Detection of IL-23 in BALF by enzyme-linked immunosorbent assay

BAL was performed using an 18-gauge needle and syringe loaded with 0.6 ml of cold PBS. The needle was inserted into the trachea, and PBS was injected and aspirated three times, slowly. About 0.4 ml of BALF was recovered from each mouse and kept on ice. Supernatant was collected after centrifugation, and aliquots were stored under −80°C for further experiments. The concentration of IL-23 in BALF was measured using a mouse IL-23 Quantikine ELISA (enzyme-linked immunosorbent assay) Kit (R&D Systems) according to the manufacturer's instructions.

Statistical analysis

Statistical tests were performed using the Prism GraphPad software (version 7.0, La Jolla, CA). Comparisons between groups were performed using Student's t tests between two groups and one-way analysis of variance (ANOVA) tests for multiple groups as appropriate. All experiments were performed at least twice independently with similar results.

SUPPLEMENTARY MATERIALS

immunology.sciencemag.org/cgi/content/full/4/41/eaaw0402/DC1

Fig. S1. Validation of BM chimeras for assessment of MR1-dependent MAIT cell activation.

Fig. S2. MAIT cell expansion after bacterial infection is impaired in *Icos*$^{-/-}$ mice.

Downloaded from http://immunology.sciencemag.org/ by guest on January 25, 2021

Fig. S3. IL-23 is required for optimal MAIT cell accumulation and activation during pulmonary infection with *L. longbeachae*.
Fig. S4. IL-23 is expressed in the lungs from hydrodynamically delivered plasmid DNA and expands MAIT cells in the lungs in combination with 5-OP-RU.
Fig. S5. MAIT cells respond directly to IL-23.
Fig. S6. IL-23 drives IL-17 production by human MAIT cells in response to 5-OP-RU.
Table S1. Raw data in Excel spreadsheet.

View/request a protocol for this paper from *Bio-protocol*.

REFERENCES AND NOTES

1. A. J. Corbett, S. B. Eckle, R. W. Birkinshaw, L. Liu, O. Patel, J. Mahony, Z. Chen, R. Reantragoon, B. Meehan, H. Cao, N. A. Williamson, R. A. Strugnell, D. Van Sinderen, J. Y. Mak, D. P. Fairlie, L. Kjer-Nielsen, J. Rossjohn, J. McCluskey, T-cell activation by transitory neo-antigens derived from distinct microbial pathways. *Nature* **509**, 361–365 (2014).
2. L. Kjer-Nielsen, O. Patel, A. J. Corbett, J. Le Nours, B. Meehan, L. Liu, M. Bhati, Z. Chen, L. Kostenko, R. Reantragoon, N. A. Williamson, A. W. Purcell, N. L. Dudek, M. J. McConville, R. A. O'Hair, G. N. Khairallah, D. I. Godfrey, D. P. Fairlie, J. Rossjohn, J. McCluskey, MR1 presents microbial vitamin B metabolites to MAIT cells. *Nature* **491**, 717–723 (2012).
3. L. Le Bourhis, L. Guerri, M. Dusseaux, E. Martin, C. Soudais, O. Lantz, Mucosal-associated invariant T cells: Unconventional development and function. *Trends Immunol.* **32**, 212–218 (2011).
4. M. Lepore, A. Kalinichenko, A. Colone, B. Paleja, A. Singhal, A. Tschumi, B. Lee, M. Poidinger, F. Zolezzi, L. Quagliata, P. Sander, E. Newell, A. Bertoletti, L. Terracciano, G. De Libero, L. Mori, Parallel T-cell cloning and deep sequencing of human MAIT cells reveal stable oligoclonal TCRβ repertoire. *Nat. Commun.* **5**, 3866 (2014).
5. R. Reantragoon, A. J. Corbett, I. G. Sakala, N. A. Gherardin, J. B. Furness, Z. Chen, S. B. Eckle, A. P. Uldrich, R. W. Birkinshaw, O. Patel, L. Kostenko, B. Meehan, K. Kedzierska, L. Liu, D. P. Fairlie, T. H. Hansen, D. I. Godfrey, J. Rossjohn, J. McCluskey, L. Kjer-Nielsen, Antigen-loaded MR1 tetramers define T cell receptor heterogeneity in mucosal-associated invariant T cells. *J. Exp. Med.* **210**, 2305–2320 (2013).
6. E. Treiner, L. Duban, S. Bahram, M. Radosavljevic, V. Wanner, F. Tilloy, P. Affaticati, S. Gilfillan, O. Lantz, Selection of evolutionarily conserved mucosal-associated invariant T cells by MR1. *Nature* **422**, 164–169 (2003).
7. F. Tilloy, E. Treiner, S. H. Park, C. Garcia, F. Lemonnier, H. de la Salle, A. Bendelac, M. Bonneville, O. Lantz, An invariant T cell receptor alpha chain defines a novel TAP-independent major histocompatibility complex class Ib-restricted α/β T cell subpopulation in mammals. *J. Exp. Med.* **189**, 1907–1921 (1999).
8. J. E. Ussher, M. Bilton, E. Attwod, J. Shadwell, R. Richardson, C. de Lara, E. Mettke, A. Kurioka, T. H. Hansen, P. Klenerman, C. B. Willberg, $CD161^{++}$ $CD8^{+}$ T cells, including the MAIT cell subset, are specifically activated by $IL\text{-}12^{+}IL\text{-}18$ in a TCR-independent manner. *Eur. J. Immunol.* **44**, 195–203 (2014).
9. C. Tastan, E. Karhan, W. Zhou, E. Fleming, A. Y. Voigt, X. Yao, L. Wang, M. Horne, L. Placek, L. Kozhaya, J. Oh, D. Unutmaz, Tuning of human MAIT cell activation by commensal bacteria species and MR1-dependent T-cell presentation. *Mucosal Immunol.* **11**, 1591–1605 (2018).
10. N. Hartmann, C. McMurtrey, M. L. Sorensen, M. E. Huber, R. Kurapova, F. T. Coleman, J. P. Mizgerd, W. Hildebrand, M. Kronenberg, D. M. Lewinsohn, M. J. Harriff, Riboflavin metabolism variation among clinical isolates of *Streptococcus pneumoniae* results in differential activation of Mucosal-associated invariant T cells. *Am. J. Respir. Cell Mol. Biol.* **58**, 767–776 (2018).
11. P. Riegert, V. Wanner, S. Bahram, Genomics, isoforms, expression, and phylogeny of the MHC class I-related MR1 gene. *J. Immunol.* **161**, 4066–4077 (1998).
12. S. Huang, S. Gilfillan, S. Kim, B. Thompson, X. Wang, A. J. Sant, D. H. Fremont, O. Lantz, T. H. Hansen, MR1 uses an endocytic pathway to activate mucosal-associated invariant T cells. *J. Exp. Med.* **205**, 1201–1211 (2008).
13. S. Huang, E. Martin, S. Kim, L. Yu, C. Soudais, D. H. Fremont, O. Lantz, T. H. Hansen, MR1 antigen presentation to mucosal-associated invariant T cells was highly conserved in evolution. *Proc. Natl. Acad. Sci. U.S.A.* **106**, 8290–8295 (2009).
14. M. Dusseaux, E. Martin, N. Serriari, I. Peguillet, V. Premel, D. Louis, M. Milder, L. Le Bourhis, C. Soudais, E. Treiner, O. Lantz, Human MAIT cells are xenobiotic-resistant, tissue-targeted, $CD161^{hi}$ IL-17-secreting T cells. *Blood* **117**, 1250–1259 (2011).
15. L. Le Bourhis, E. Martin, I. Peguillet, A. Guihot, N. Froux, M. Core, E. Levy, M. Dusseaux, V. Meyssonnier, V. Premel, C. Ngo, B. Riteau, L. Duban, D. Robert, S. Huang, M. Rottman, C. Soudais, O. Lantz, Antimicrobial activity of mucosal-associated invariant T cells. *Nat. Immunol.* **11**, 701–708 (2010).
16. E. Martin, E. Treiner, L. Duban, L. Guerri, H. Laude, C. Toly, V. Premel, A. Devys, I. C. Moura, F. Tilloy, S. Cherif, G. Vera, S. Latour, C. Soudais, O. Lantz, Stepwise development of MAIT cells in mouse and human. *PLOS Biol.* **7**, e1000054 (2009).
17. H.-F. Koay, N. A. Gherardin, A. Enders, L. Loh, L. K. Mackay, C. F. Almeida, B. E. Russ, C. A. Nold-Petry, M. F. Nold, S. Bedoui, Z. Chen, A. J. Corbett, S. B. Eckle, B. Meehan, Y. d'Udekem, I. E. Konstantinov, M. Lappas, L. Liu, C. C. Goodnow, D. P. Fairlie, J. Rossjohn, M. M. Chong, K. Kedzierska, S. P. Berzins, G. T. Belz, J. McCluskey, A. P. Uldrich, D. I. Godfrey, D. G. Pellicci, A three-stage intrathymic development pathway for the mucosal-associated invariant T cell lineage. *Nat. Immunol.* **17**, 1300–1311 (2016).
18. M. C. Gold, S. Cerri, S. Smyk-Pearson, M. E. Cansler, T. M. Vogt, J. Delepine, E. Winata, G. M. Swarbrick, W. J. Chua, Y. Y. Yu, O. Lantz, M. S. Cook, M. D. Null, D. B. Jacoby, M. J. Harriff, D. A. Lewinsohn, T. H. Hansen, D. M. Lewinsohn, Human mucosal associated invariant T cells detect bacterially infected cells. *PLOS Biol.* **8**, e1000407 (2010).
19. Z. Chen, H. Wang, C. D'Souza, S. Sun, L. Kostenko, S. B. G. Eckle, B. S. Meehan, D. C. Jackson, R. A. Strugnell, H. Cao, N. Wang, D. P. Fairlie, L. Liu, D. I. Godfrey, J. Rossjohn, J. McCluskey, A. J. Corbett, 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 (2017).
20. H. Wang, C. D'Souza, X. Y. Lim, L. Kostenko, T. J. Pediongco, S. B. G. Eckle, B. S. Meehan, M. Shi, N. Wang, S. Li, L. Liu, J. Y. W. Mak, D. P. Fairlie, Y. Iwakura, J. M. Gunnersen, A. W. Stent, D. I. Godfrey, J. Rossjohn, G. P. Westall, L. Kjer-Nielsen, R. A. Strugnell, J. McCluskey, A. J. Corbett, T. S. C. Hinks, Z. Chen, MAIT cells protect against pulmonary *Legionella longbeachae* infection. *Nat. Commun.* **9**, 3350 (2018).
21. C. K. Slichter, A. McDavid, H. W. Miller, G. Finak, B. J. Seymour, J. P. McNevin, G. Diaz, J. L. Czartoski, M. J. McElrath, R. Gottardo, M. Prlic, Distinct activation thresholds of human conventional and innate-like memory T cells. *JCI Insight* **1**, e86292 (2016).
22. E. Gracey, Z. Qaiyum, I. Almaghlouth, D. Lawson, S. Karki, N. Avvaru, Z. Zhang, Y. Yao, V. Ranganathan, Y. Baglaenko, R. D. Inman, IL-7 primes IL-17 in mucosal-associated invariant T (MAIT) cells, which contribute to the Th17-axis in ankylosing spondylitis. *Ann. Rheum. Dis.* **75**, 2124–2132 (2016).
23. J. Jo, A. T. Tan, J. E. Ussher, E. Sandalova, X.-Z. Tang, A. Tan-Garcia, N. To, M. Hong, A. Chia, U. S. Gill, P. T. Kennedy, K. C. Tan, K. H. Lee, G. De Libero, A. J. Gehring, C. B. Willberg, P. Klenerman, A. Bertoletti, Toll-like receptor 8 agonist and bacteria trigger potent activation of innate immune cells in human liver. *PLOS Pathog.* **10**, e1004210 (2014).
24. A. Sattler, C. Dang-Heine, P. Reinke, N. Babel, IL-15 dependent induction of IL-18 secretion as a feedback mechanism controlling human MAIT-cell effector functions. *Eur. J. Immunol.* **45**, 2286–2298 (2015).
25. J. C. Wallington, A. P. Williams, K. J. Staples, T. M. A. Wilkinson, IL-12 and IL-7 synergize to control mucosal-associated invariant T-cell cytotoxic responses to bacterial infection. *J. Allergy Clin. Immunol.* **141**, 2182–2195.e6 (2018).
26. W.-J. Chua, S. M. Truscott, C. S. Eickhoff, A. Blazevic, D. F. Hoft, T. H. Hansen, Polyclonal mucosa-associated invariant T cells have unique innate functions in bacterial infection. *Infect. Immun.* **80**, 3256–3267 (2012).
27. C. Soudais, F. Samassa, M. Sarkis, L. L. Bourhis, S. Bessoles, D. Blanot, M. Hervé, F. Schmidt, D. Mengin-Lecreulx, O. Lantz, In vitro and in vivo analysis of the gram-negative bacteria–derived riboflavin precursor derivatives activating mouse MAIT cells. *J. Immunol.* **194**, 4641–4649 (2015).
28. M. J. Harriff, M. E. Cansler, K. G. Toren, E. T. Canfield, S. Kwak, M. C. Gold, D. M. Lewinsohn, Human lung epithelial cells contain *Mycobacterium tuberculosis* in a late endosomal vacuole and are efficiently recognized by $CD8^{+}$ T cells. *PLOS ONE* **9**, e97515 (2014).
29. H. C. Jeffery, B. van Wilgenburg, A. Kurioka, K. Parekh, K. Stirling, S. Roberts, E. E. Dutton, S. Hunter, D. Geh, M. K. Braitch, J. Rajanayagam, T. Iqbal, T. Pinkney, R. Brown, D. R. Withers, D. H. Adams, P. Klenerman, Y. H. Oo, Biliary epithelium and liver B cells exposed to bacteria activate intrahepatic MAIT cells through MR1. *J. Hepatol.* **64**, 1118–1127 (2016).
30. K. G. Anderson, H. Sung, C. N. Skon, L. Lefrancois, A. Deisinger, V. Vezys, D. Masopust, Cutting edge: Intravascular staining redefines lung CD8 T cell responses. *J. Immunol.* **189**, 2702–2706 (2012).
31. A. Rahimpour, H. F. Koay, A. Enders, R. Clanchy, S. B. G. Eckle, B. Meehan, Z. Chen, B. Whittle, L. Liu, D. P. Fairlie, C. C. Goodnow, J. McCluskey, J. Rossjohn, A. P. Uldrich, D. G. Pellicci, D. I. Godfrey, Identification of phenotypically and functionally heterogeneous mouse Mucosal-associated invariant T cells using MR1 tetramers. *J. Exp. Med.* **20**, 1095–1108 (2015).
32. N. Seach, L. Guerri, L. Le Bourhis, Y. Mburu, Y. Cui, S. Bessoles, C. Soudais, O. Lantz, Double-positive thymocytes select mucosal-associated invariant T cells. *J. Immunol.* **191**, 6002–6009 (2013).
33. D. Kreisel, S. B. Richardson, W. Li, X. Lin, C. G. Kornfeld, S. Sugimoto, C. S. Hsieh, A. E. Gelman, A. S. Krupnick, Cutting edge: MHC class II expression by pulmonary nonhematopoietic cells plays a critical role in controlling local inflammatory responses. *J. Immunol.* **185**, 3809–3813 (2010).
34. M. Gereke, S. Jung, J. Buer, D. Bruder, Alveolar type II epithelial cells present antigen to $CD4^{+}$ T cells and induce $Foxp3^{+}$ regulatory T cells. *Am. J. Respir. Crit. Care Med.* **179**, 344–355 (2009).

Downloaded from http://immunology.sciencemag.org/ by guest on January 25, 2021

35. J. E. Galan, R. Curtiss III, Cloning and molecular characterization of genes whose products allow *Salmonella typhimurium* to penetrate tissue culture cells. *Proc. Natl. Acad. Sci. U.S.A.* **86**, 6383–6387 (1989).
36. O. L. C. Wijburg, N. van Rooijen, R. A. Strugnell, Induction of $CD8^+$ T lymphocytes by *Salmonella typhimurium* is independent of Salmonella pathogenicity island 1-mediated host cell death. *J. Immunol.* **169**, 3275–3283 (2002).
37. R. Reantragoon, L. Kjer-Nielsen, O. Patel, Z. Chen, P. T. Illing, M. Bhati, L. Kostenko, M. Bharadwaj, B. Meehan, T. H. Hansen, D. I. Godfrey, J. Rossjohn, J. McCluskey, Structural insight into MR1-mediated recognition of the mucosal associated invariant T cell receptor. *J. Exp. Med.* **209**, 761–774 (2012).
38. J. Liu, R. R. Brutkiewicz, The Toll-like receptor 9 signalling pathway regulates MR1-mediated bacterial antigen presentation in B cells. *Immunology* **152**, 232–242 (2017).
39. T. Korn, E. Bettelli, M. Oukka, V. K. Kuchroo, IL-17 and Th17 cells. *Annu. Rev. Immunol.* **27**, 485–517 (2009).
40. S. J. Szabo, S. T. Kim, G. L. Costa, X. Zhang, C. G. Fathman, L. H. Glimcher, A novel transcription factor, T-bet, directs Th1 lineage commitment. *Cell* **100**, 655–669 (2000).
41. A. Kurioka, B. van Wilgenburg, R. R. Javan, R. Hoyle, A. J. van Tonder, C. L. Harrold, T. Leng, L. J. Howson, D. Shepherd, V. Cerundolo, A. B. Brueggemann, P. Klenerman, Diverse *Streptococcus pneumoniae* strains drive a mucosal-associated invariant T-cell response through major histocompatibility complex class I-related molecule-dependent and cytokine-driven pathways. *J. Infect. Dis.* **217**, 988–999 (2018).
42. F. Mattner, J. Magram, J. Ferrante, P. Launois, K. Di Padova, R. Behin, M. K. Gately, J. A. Louis, G. Alber, Genetically resistant mice lacking interleukin-12 are susceptible to infection with *Leishmania major* and mount a polarized Th2 cell response. *Eur. J. Immunol.* **26**, 1553–1559 (1996).
43. D. J. Cua, J. Sherlock, Y. Chen, C. A. Murphy, B. Joyce, B. Seymour, L. Lucian, W. To, S. Kwan, T. Churakova, S. Zurawski, M. Wiekowski, S. A. Lira, D. Gorman, R. A. Kastelein, J. D. Sedgwick, Interleukin-23 rather than interleukin-12 is the critical cytokine for autoimmune inflammation of the brain. *Nature* **421**, 744–748 (2003).
44. J. Magram, S. E. Connaughton, R. R. Warrier, D. M. Carvajal, C. Y. Wu, J. Ferrante, C. Stewart, U. Sarmiento, D. A. Faherty, M. K. Gately, IL-12-deficient mice are defective in IFNγ production and type 1 cytokine responses. *Immunity* **4**, 471–481 (1996).
45. N. Ghilardi, N. Kljavin, Q. Chen, S. Lucas, A. L. Gurney, F. J. de Sauvage, Compromised humoral and delayed-type hypersensitivity responses in IL-23-deficient mice. *J. Immunol.* **172**, 2827–2833 (2004).
46. L. E. Harrington, R. D. Hatton, P. R. Mangan, H. Turner, T. L. Murphy, K. M. Murphy, C. T. Weaver, Interleukin 17-producing $CD4^+$ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. *Nat. Immunol.* **6**, 1123–1132 (2005).
47. S. Aggarwal, N. Ghilardi, M.-H. Xie, F. J. de Sauvage, A. L. Gurney, Interleukin-23 promotes a distinct CD4 T cell activation state characterized by the production of interleukin-17. *J. Biol. Chem.* **278**, 1910–1914 (2003).
48. H. Park, Z. Li, X. O. Yang, S. H. Chang, R. Nurieva, Y. H. Wang, Y. Wang, L. Hood, Z. Zhu, Q. Tian, C. Dong, A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin 17. *Nat. Immunol.* **6**, 1133–1141 (2005).
49. C. S. Hsieh, S. E. Macatonia, C. S. Tripp, S. F. Wolf, A. O'Garra, K. M. Murphy, Development of TH1 CD4+ T cells through IL-12 produced by Listeria-induced macrophages. *Science* **260**, 547–549 (1993).
50. C. Heufler, F. Koch, U. Stanzl, G. Topar, M. Wysocka, G. Trinchieri, A. Enk, R. M. Steinman, N. Romani, G. Schuler, Interleukin-12 is produced by dendritic cells and mediates T helper 1 development as well as interferon-γ production by T helper 1 cells. *Eur. J. Immunol.* **26**, 659–668 (1996).
51. R. Manetti, P. Parronchi, M. G. Giudizi, M. P. Piccinni, E. Maggi, G. Trinchieri, S. Romagnani, Natural killer cell stimulatory factor (interleukin 12 [IL-12]) induces T helper type 1 (Th1)-specific immune responses and inhibits the development of IL-4-producing Th cells. *J. Exp. Med.* **177**, 1199–1204 (1993).
52. C. S. Tripp, S. F. Wolf, E. R. Unanue, Interleukin 12 and tumor necrosis factor alpha are costimulators of interferon gamma production by natural killer cells in severe combined immunodeficiency mice with listeriosis, and interleukin 10 is a physiologic antagonist. *Proc. Natl. Acad. Sci. U.S.A.* **90**, 3725–3729 (1993).
53. R. A. Seder, R. Gazzinelli, A. Sher, W. E. Paul, Interleukin 12 acts directly on CD4+ T cells to enhance priming for interferon gamma production and diminishes interleukin 4 inhibition of such priming. *Proc. Natl. Acad. Sci. U.S.A.* **90**, 10188–10192 (1993).
54. A. J. McKnight, G. J. Zimmer, I. Fogelman, S. F. Wolf, A. K. Abbas, Effects of IL-12 on helper T cell-dependent immune responses in vivo. *J. Immunol.* **152**, 2172–2179 (1994).
55. T. Korn, E. Bettelli, W. Gao, A. Awasthi, A. Jager, T. B. Strom, M. Oukka, V. K. Kuchroo, IL-21 initiates an alternative pathway to induce proinflammatory T_H17 cells. *Nature* **448**, 484–487 (2007).
56. V. Upadhyay, V. Poroyko, T.-J. Kim, S. Devkota, S. Fu, D. Liu, A. V. Tumanov, E. P. Koroleva, L. Deng, C. Nagler, E. B. Chang, H. Tang, Y.-X. Fu, Lymphotoxin regulates commensal responses to enable diet-induced obesity. *Nat. Immunol.* **13**, 947–953 (2012).
57. F. Liu, Y. K. Song, D. Liu, Hydrodynamics-based transfection in animals by systemic administration of plasmid DNA. *Gene Ther.* **6**, 1258–1266 (1999).
58. A. Awasthi, L. Riol-Blanco, A. Jager, T. Korn, C. Pot, G. Galileos, E. Bettelli, V. K. Kuchroo, M. Oukka, Cutting edge: IL-23 receptor gfp reporter mice reveal distinct populations of IL-17-producing cells. *J. Immunol.* **182**, 5904–5908 (2009).
59. M. J. Harriff, E. Karamooz, A. Burr, W. F. Grant, E. T. Canfield, M. L. Sorensen, L. F. Moita, D. M. Lewinsohn, Endosomal MR1 trafficking plays a key role in presentation of *Mycobacterium tuberculosis* ligands to MAIT cells. *PLOS Pathog.* **12**, e1005524 (2016).
60. A. S. Brown, C. Yang, K. Y. Fung, A. Bachem, D. Bourges, S. Bedoui, E. L. Hartland, I. R. van Driel, Cooperation between monocyte-derived cells and lymphoid cells in the acute response to a bacterial lung pathogen. *PLOS Pathog.* **12**, e1005691 (2016).
61. P. J. Hume, V. Singh, A. C. Davidson, V. Koronakis, Swiss army pathogen: The *Salmonella* entry toolkit. *Front. Cell. Infect. Microbiol.* **7**, 348 (2017).
62. A. W. M. van der Velden, M. K. Copass, M. N. Starnbach, *Salmonella* inhibit T cell proliferation by a direct, contact-dependent immunosuppressive effect. *Proc. Natl. Acad. Sci. U.S.A.* **102**, 17769–17774 (2005).
63. N. Lapaque, J. L. Hutchinson, D. C. Jones, S. Méresse, D. W. Holden, J. Trowsdale, A. P. Kelly, *Salmonella* regulates polyubiquitination and surface expression of MHC class II antigens. *Proc. Natl. Acad. Sci. U.S.A.* **106**, 14052–14057 (2009).
64. E. Bayer-Santos, C. H. Durkin, L. A. Rigano, A. Kupz, E. Alix, O. Cerny, E. Jennings, M. Liu, A. S. Ryan, N. Lapaque, S. H. E. Kaufmann, D. W. Holden, The *Salmonella* effector SteD mediates MARCH8-dependent ubiquitination of MHC II molecules and inhibits T cell activation. *Cell Host Microbe* **20**, 584–595 (2016).
65. C. Dong, A. E. Juedes, U.-A. Temann, S. Shresta, J. P. Allison, N. H. Ruddle, R. A. Flavell, ICOS co-stimulatory receptor is essential for T-cell activation and function. *Nature* **409**, 97–101 (2001).
66. D. J. Wikenheiser, J. S. Stumhofer, ICOS co-stimulation: Friend or foe? *Front. Immunol.* **7**, 304 (2016).
67. S. Khayyamian, A. Hutloff, K. Buchner, M. Grafe, V. Henn, R. A. Kroczek, H. W. Mages, ICOS-ligand, expressed on human endothelial cells, costimulates Th1 and Th2 cytokine secretion by memory $CD4^+$ T cells. *Proc. Natl. Acad. Sci. U.S.A.* **99**, 6198–6203 (2002).
68. H. W. Mages, A. Hutloff, C. Heuck, K. Buchner, H. Himmelbauer, F. Oliveri, R. A. Kroczek, Molecular cloning and characterization of murine ICOS and identification of B7h as ICOS ligand. *Eur. J. Immunol.* **30**, 1040–1047 (2000).
69. V. Ling, P. W. Wu, H. F. Finnerty, K. M. Bean, V. Spaulding, L. A. Fouser, J. P. Leonard, S. E. Hunter, R. Zollner, J. L. Thomas, J. S. Miyashiro, K. A. Jacobs, M. Collins, Cutting edge: Identification of GL50, a novel B7-like protein that functionally binds to ICOS receptor. *J. Immunol.* **164**, 1653–1657 (2000).
70. M. M. Swallow, J. J. Wallin, W. C. Sha, B7h, a novel costimulatory homolog of B7.1 and B7.2, is induced by TNFα. *Immunity* **11**, 423–432 (1999).
71. G. Richter, M. Hayden-Ledbetter, M. Irgang, J. A. Ledbetter, J. Westermann, I. Korner, K. Daemen, E. A. Clark, A. Aicher, A. Pezzutto, Tumor necrosis factor-alpha regulates the expression of inducible costimulator receptor ligand on $CD34^+$ progenitor cells during differentiation into antigen presenting cells. *J. Biol. Chem.* **276**, 45686–45693 (2001).
72. C. H. June, J. A. Ledbetter, M. M. Gillespie, T. Lindsten, C. B. Thompson, T-cell proliferation involving the CD28 pathway is associated with cyclosporine-resistant interleukin 2 gene expression. *Mol. Cell. Biol.* **7**, 4472–4481 (1987).
73. A. Bendelac, P. B. Savage, L. Teyton, The biology of NKT cells. *Annu. Rev. Immunol.* **25**, 297–336 (2007).
74. J. Jiang, Z. Cao, W. Shan, H. Liu, X. Cheng, 4-1BB expression on MAIT cells is associated with enhanced IFN-γ production and depends on IL-2. *Cell. Immunol.* **328**, 58–69 (2018).
75. M. H. Nelson, S. Kundimi, J. S. Bowers, C. E. Rogers, L. W. Huff, K. M. Schwartz, K. Thyagarajan, E. C. Little, S. Mehrotra, D. J. Cole, M. P. Rubinstein, C. M. Paulos, The inducible costimulator augments Tc17 cell responses to self and tumor tissue. *J. Immunol.* **194**, 1737–1747 (2015).
76. L. Zhou, I. I. Ivanov, R. Spolski, R. Min, K. Shenderov, T. Egawa, D. E. Levy, W. J. Leonard, D. R. Littman, IL-6 programs T_H-17 cell differentiation by promoting sequential engagement of the IL-21 and IL-23 pathways. *Nat. Immunol.* **8**, 967–974 (2007).
77. L. Yang, D. E. Anderson, C. Baecher-Allan, W. D. Hastings, E. Bettelli, M. Oukka, V. K. Kuchroo, D. A. Hafler, IL-21 and TGF-β are required for differentiation of human T_H17 cells. *Nature* **454**, 350–352 (2008).
78. R. P. Wilson, M. L. Ives, G. Rao, A. Lau, K. Payne, M. Kobayashi, P. D. Arkwright, J. Peake, M. Wong, S. Adelstein, J. M. Smart, M. A. French, D. A. Fulcher, C. Picard, J. Bustamante, S. Boisson-Dupuis, P. Gray, P. Stepensky, K. Warnatz, A. F. Freeman, J. Rossjohn, J. McCluskey, S. M. Holland, J. L. Casanova, G. Uzel, C. S. Ma, S. G. Tangye, E. K. Deenick, STAT3 is a critical cell-intrinsic regulator of human unconventional T cell numbers and function. *J. Exp. Med.* **212**, 855–864 (2015).
79. L. J. Walker, Y.-H. Kang, M. O. Smith, H. Tharmalingham, N. Ramamurthy, V. M. Fleming, N. Sahgal, A. Leslie, Y. Oo, A. Geremia, T. J. Scriba, W. A. Hanekom, G. M. Lauer, O. Lantz,

Downloaded from http://immunology.sciencemag.org/ by guest on January 25, 2021

D. H. Adams, F. Powrie, E. Barnes, P. Klenerman, Human MAIT and CD8αα cells develop from a pool of type-17 precommitted CD8+ T cells. *Blood* **119**, 422–433 (2012).
80. G. R. Yannam, T. Gutti, L. Y. Poluektova, IL-23 in infections, inflammation, autoimmunity and cancer: Possible role in HIV-1 and AIDS. *J. Neuroimmune Pharmacol.* **7**, 95–112 (2012).
81. S. L. Gaffen, R. Jain, A. V. Garg, D. J. Cua, The IL-23-IL-17 immune axis: From mechanisms to therapeutic testing. *Nat. Rev. Immunol.* **14**, 585–600 (2014).
82. L. Wassink, P. L. Vieira, H. H. Smits, G. A. Kingsbury, A. J. Coyle, M. L. Kapsenberg, E. A. Wierenga, ICOS expression by activated human Th cells is enhanced by IL-12 and IL-23: Increased ICOS expression enhances the effector function of both Th1 and Th2 cells. *J. Immunol.* **173**, 1779–1786 (2004).
83. I. I. Ivanov, B. S. McKenzie, L. Zhou, C. E. Tadokoro, A. Lepelley, J. J. Lafaille, D. J. Cua, D. R. Littman, The orphan nuclear receptor RORγt directs the differentiation program of proinflammatory IL-17+ T helper cells. *Cell* **126**, 1121–1133 (2006).
84. E. Jesteadt, I. Zhang, H. Yu, A. Meierovics, W.-J. Chua Yankelevich, S. Cowley, Interleukin-18 is critical for mucosa-associated invariant T cell gamma interferon responses to *Francisella* species in vitro but not in vivo. *Infect. Immun.* **86**, e00117-18 (2018).
85. I. G. Sakala, L. Kjer-Nielsen, C. S. Eickhoff, X. Wang, A. Blazevic, L. Liu, D. P. Fairlie, J. Rossjohn, J. McCluskey, D. H. Fremont, T. H. Hansen, D. F. Hoft, Functional heterogeneity and antimycobacterial effects of mouse mucosal-associated invariant T cells specific for riboflavin metabolites. *J. Immunol.* **195**, 587–601 (2015).
86. P. Georgel, M. Radosavljevic, C. Macquin, S. Bahram, The non-conventional MHC class I MR1 molecule controls infection by *Klebsiella pneumoniae* in mice. *Mol. Immunol.* **48**, 769–775 (2011).
87. A. Meierovics, W. J. Yankelevich, S. C. Cowley, MAIT cells are critical for optimal mucosal immune responses during in vivo pulmonary bacterial infection. *Proc. Natl. Acad. Sci. U.S.A.* **110**, E3119–E3128 (2013).
88. O. Rouxel, J. Da Silva, L. Beaudoin, I. Nel, C. Tard, L. Cagninacci, B. Kiaf, M. Oshima, M. Diedisheim, M. Salou, A. Corbett, J. Rossjohn, J. McCluskey, R. Scharfmann, M. Battaglia, M. Polak, O. Lantz, J. Beltrand, A. Lehuen, Cytotoxic and regulatory roles of mucosal-associated invariant T cells in type 1 diabetes. *Nat. Immunol.* **18**, 1321–1331 (2017).
89. T. S. C. Hinks, E. Marchi, M. Jabeen, M. Olshansky, A. Kurioka, T. J. Pediongco, B. S. Meehan, L. Kostenko, S. J. Turner, A. J. Corbett, Z. Chen, P. Klenerman, J. McCluskey, Activation and in vivo evolution of the MAIT cell transcriptome in mice and humans reveals tissue repair functionality. *Cell Rep.* **28**, 3249–3262.e5 (2019).
90. S. J. Gebran, Y. Yamamoto, C. Newton, T. W. Klein, H. Friedman, Inhibition of *Legionella pneumophila* growth by gamma interferon in permissive A/J mouse macrophages: Role of reactive oxygen species, nitric oxide, tryptophan, and iron(III). *Infect. Immun.* **62**, 3197–3205 (1994).
91. S. J. Skerrett, T. R. Martin, Intratracheal interferon-gamma augments pulmonary defenses in experimental legionellosis. *Am. J. Respir. Crit. Care Med.* **149**, 50–58 (1994).
92. Y. Kimizuka, S. Kimura, T. Saga, M. Ishii, N. Hasegawa, T. Betsuyaku, Y. Iwakura, K. Tateda, K. Yamaguchi, Roles of interleukin-17 in an experimental *Legionella pneumophila* pneumonia model. *Infect. Immun.* **80**, 1121–1127 (2012).
93. I. Kawachi, J. Maldonado, C. Strader, S. Gilfillan, MR1-restricted Vα19i mucosal-associated invariant T cells are innate T cells in the gut lamina propria that provide a rapid and diverse cytokine response. *J. Immunol.* **176**, 1618–1627 (2006).
94. J. Y. W. Mak, W. Xu, R. C. Reid, A. J. Corbett, B. S. Meehan, H. Wang, Z. Chen, J. Rossjohn, J. McCluskey, L. Liu, D. P. Fairlie, Stabilizing short-lived Schiff base derivatives of 5-aminouracils that activate mucosal-associated invariant T cells. *Nat. Commun.* **8**, 14599 (2017).
95. O. Patel, L. Kjer-Nielsen, J. Le Nours, S. B. Eckle, R. Birkinshaw, T. Beddoe, A. J. Corbett, L. Liu, J. J. Miles, B. Meehan, R. Reantragoon, M. L. Sandoval-Romero, L. C. Sullivan, A. G. Brooks, Z. Chen, D. P. Fairlie, J. McCluskey, J. Rossjohn, Recognition of vitamin B metabolites by mucosal-associated invariant T cells. *Nat. Commun.* **4**, 2142 (2013).
96. C. M. Rosenberger, R. L. Podyminogin, P. S. Askovich, G. Navarro, S. M. Kaiser, C. J. Sanders, J. L. McClaren, V. C. Tam, P. Dash, J. G. Noonan, B. G. Jones, S. L. Surman, J. J. Peschon, A. H. Diercks, J. L. Hurwitz, P. C. Doherty, P. G. Thomas, A. Aderem, Characterization of innate responses to influenza virus infection in a novel lung type I epithelial cell model. *J. Gen. Virol.* **95**, 350–362 (2014).
97. M. L. Belladonna, J.-C. Renauld, R. Bianchi, C. Vacca, F. Fallarino, C. Orabona, M. C. Fioretti, U. Grohmann, P. Puccetti, IL-23 and IL-12 have overlapping, but distinct, effects on murine dendritic cells. *J. Immunol.* **168**, 5448–5454 (2002).
98. H. Herweijer, J. A. Wolff, Progress and prospects: Naked DNA gene transfer and therapy. *Gene Ther.* **10**, 453–458 (2003).
99. A. V. Tumanov, E. P. Koroleva, X. Guo, Y. Wang, A. Kruglov, S. Nedospasov, Y.-X. Fu, Lymphotoxin controls the IL-22 protection pathway in gut innate lymphoid cells during mucosal pathogen challenge. *Cell Host Microbe* **10**, 44–53 (2011).

Acknowledgments: We thank P. Cao for advice on statistical analysis of data. We thank B. Becher, University of Zürich, Institute of Experimental Immunology, for provision of the IL-23–Ig construct and V. Kuchroo, Harvard Medical School and Brigham and Women's Hospital, for the IL-23R^{GFP} reporter mice. We thank H. Newton for provision of *L. longbeachae* NSW150 and E. Hartland (Monash University) for provision of *Il-12p35*$^{-/-}$, *Il-12p40*$^{-/-}$, and *Il-12p19*$^{-/-}$ mice. **Funding:** We acknowledge the support of the National Health and Medical Research Council of Australia (program grants 1113293 and 1092262 and project grant 1120467) and the Australian Research Council (Discovery Project DP170104321). A.J.C. was supported by an ARC Future Fellowship FT160100083. D.P.F. was supported by an NHMRC Senior Principal Research Fellowship 1117017. J.R. was supported by an ARC Australian Laureate Fellowship FT160100049. H.W. was supported by a Melbourne International Engagement Award (The University of Melbourne). C.D. was supported by a Melbourne International Research Scholarship and a Melbourne International Fee Remission Scholarship (The University of Melbourne). **Author contributions:** H.W., L.K.-N., M.S., C.D., T.J.P., H.C., L.K., X.Y.L., B.W., T.Z., and Z.C. designed and performed experiments. S.B.G.E., B.S.M., J.Y.W.M., D.P.F., M.W.L.T., J.R., D.Y., B.F.d.S.G., G.L., and L.L. provided vital reagents and expertise. J.M., R.A.S., Z.C., H.W., and A.J.C. designed and analyzed experiments, coordinated the study, and wrote the paper. All authors edited the paper. **Competing interests:** L.K.-N., A.J.C., Z.C., S.B.G.E., D.P.F., J.M., J.R., and J.M. are inventors on patents (WO2014/005194 and WO2015/149130) describing MR1 tetramers and MR1 ligands. The other authors declare that they have no competing interests. **Data and materials availability:** Reagents developed for the stimulation and detection of MAIT cells are available from the authors through a material transfer agreement. All data needed to evaluate the conclusions in the paper are present in the paper or the Supplementary Materials.

Submitted 20 November 2018
Resubmitted 9 May 2019
Accepted 18 October 2019
Published 15 November 2019
10.1126/sciimmunol.aaw0402

Citation: H. Wang, L. Kjer-Nielsen, M. Shi, C. D'Souza, T. J. Pediongco, H. Cao, L. Kostenko, X. Y. Lim, S. B. G. Eckle, B. S. Meehan, T. Zhu, B. Wang, Z. Zhao, J. Y. W. Mak, D. P. Fairlie, M. W. L. Teng, J. Rossjohn, D. Yu, B. Fazekas de St Groth, G. Lovrecz, L. Lu, J. McCluskey, R. A. Strugnell, A. J. Corbett, Z. Chen, IL-23 costimulates antigen-specific MAIT cell activation and enables vaccination against bacterial infection. *Sci. Immunol.* **4**, eaaw0402 (2019).

Downloaded from http://immunology.sciencemag.org/ by guest on January 25, 2021

Science Immunology

IL-23 costimulates antigen-specific MAIT cell activation and enables vaccination against bacterial infection

Huimeng Wang, Lars Kjer-Nielsen, Mai Shi, Criselle D'Souza, Troi J. Pediongco, Hanwei Cao, Lyudmila Kostenko, Xin Yi Lim, Sidonia B. G. Eckle, Bronwyn S. Meehan, Tianyuan Zhu, Bingjie Wang, Zhe Zhao, Jeffrey Y. W. Mak, David P. Fairlie, Michele W. L. Teng, Jamie Rossjohn, Di Yu, Barbara Fazekas de St Groth, George Lovrecz, Louis Lu, James McCluskey, Richard A. Strugnell, Alexandra J. Corbett and Zhenjun Chen

Sci. Immunol. **4**, eaaw0402.
DOI: 10.1126/sciimmunol.aaw0402

In vivo costimulators for MAIT cells

Mucosal-associated invariant T (MAIT) cells contribute to immune defense against a broad range of pathogens through their ability to recognize microbial riboflavin metabolites presented by the MR1 class I MHC molecule. Wang *et al.* examined the role that costimulatory signals play in supporting in vivo MAIT cell activation and expansion in mice. Both the inducible T cell costimulator (ICOS) and interleukin-23 (IL-23) costimulatory pathways support MAIT cell expansion and thereby enhance control of infection. A prophylactic vaccine based on combining exogenous IL-23 and a potent MAIT ligand augmented host control of a subsequent pulmonary *Legionella* infection. These studies pinpoint relevant host costimulatory pathways that can be targeted to enhance MAIT function in antimicrobial defense.

ARTICLE TOOLS http://immunology.sciencemag.org/content/4/41/eaaw0402

SUPPLEMENTARY MATERIALS http://immunology.sciencemag.org/content/suppl/2019/11/11/4.41.eaaw0402.DC1

REFERENCES This article cites 99 articles, 40 of which you can access for free
http://immunology.sciencemag.org/content/4/41/eaaw0402#BIBL

Use of this article is subject to the Terms of Service

Science Immunology (ISSN 2470-9468) is published by the American Association for the Advancement of Science, 1200 New York Avenue NW, Washington, DC 20005. The title *Science Immunology* is a registered trademark of AAAS.

Copyright © 2019 The Authors, some rights reserved; exclusive licensee American Association for the Advancement of Science. No claim to original U.S. Government Works

Downloaded from http://immunology.sciencemag.org/ by guest on January 25, 2021