MAIT cells contribute to protection against lethal influenza infection in vivo

Bonnie van Wilgenburg1,2, Liyen Loh1, Zhenjun Chen1, Troi J. Pediongco1, Huimeng Wang1, Mai Shi1, Zhe Zhao1, Marios Koutsakos1, Simone Nüssing1, Sneha Sant1, Zhongfang Wang1, Criselle D’Souza1, Xiaoxiao Jia1, Catarina F. Almeida1,3, Lyudmila Kostenko1, Sidonia B.G. Eckle1, Bronwyn S. Meehan1, Axel Kallies1, Dale I. Godfrey1,3, Patrick C. Reading1, Alexandra J. Corbett1, James McCluskey1, Paul Klenerman2, Katherine Kedzierska1 & Timothy S.C. Hinks1,4

Mucosal associated invariant T (MAIT) cells are evolutionarily-conserved, innate-like lymphocytes which are abundant in human lungs and can contribute to protection against pulmonary bacterial infection. MAIT cells are also activated during human viral infections, yet it remains unknown whether MAIT cells play a significant protective or even detrimental role during viral infections in vivo. Using murine experimental challenge with two strains of influenza A virus, we show that MAIT cells accumulate and are activated early in infection, with upregulation of CD25, CD69 and Granzyme B, peaking at 5 days post-infection. Activation is modulated via cytokines independently of MR1. MAIT cell-deficient MR1−/− mice show enhanced weight loss and mortality to severe (H1N1) influenza. This is ameliorated by prior adoptive transfer of pulmonary MAIT cells in both immunocompetent and immunodeficient RAG2−/−γC−/− mice. Thus, MAIT cells contribute to protection during respiratory viral infections, and constitute a potential target for therapeutic manipulation.
Mucosal associated invariant T (MAIT) cells are an abundant and evolutionarily-conserved class of innate-like lymphocytes, which comprise up to 10% of human peripheral blood and respiratory mucosal T cells. MAIT cell development and antigen (Ag)-specific activation are restricted by the monomorphic \( \beta2 \)-microglobulin-associated molecule MHC related protein-1 (MR1). MAIT cells express a semi-invariant \( \alpha \beta \) T cell receptor (TCR) shown to recognise metabolic derivatives of highly-conserved riboflavin biosynthetic pathways, that are expressed by a wide range of bacteria, mycobacteria and yeasts. These molecules are usually short-lived, but are stabilised by MR1, and include 5-(2-oxopropylideneamino)-6-D-ribitylaminouracil (5-OP-RU), which is a potent MAIT cell ligand, and can be loaded onto MR1 to form specific tetramers to track human and murine MAIT cells.

Consistent with these observations, we and others have shown that MAIT cells contribute to protection in vivo against intracellular bacteria including Francisella tularensis, Legionella longbeachae, and mycobacteria, whilst in human vitro data suggest they may contribute also to protection against a variety of other respiratory bacterial pathogens, including Haemophilus influenzae and Streptococcus pneumoniae.

Although this MR1-TCR dependent activation appears specific for riboflavin-expressing prokaryotic pathogens, there is emerging evidence that MAIT cells have the potential also to be involved in immune responses to viruses. MAIT cells have constitutively high surface expression of the interleukin (IL)-18 receptor and IL-12 receptor, and we have shown they can be activated by IL-18 synergistically with IL-12 or IL-15 or type I interferons (IFN) to induce expression of IFN-\( \gamma \) and Granzyme B. These responses are MR1 and TCR independent. These phenotypically innate features of MAIT cells, in common with invariant natural killer T cells (iNKT), are believed to drive by expression of the master transcription factor promyelocytic leukaemia zinc finger protein (PLZF).

We have previously shown that peripheral MAIT cells are activated in vivo during human infections with influenza A virus (IAV), dengue virus and hepatitis C, whilst in vitro these viruses activate MAIT cells in an IL-18 dependent manner. Moreover, we showed that activation correlated with disease activity in IAV infection and reduced peripheral blood MAIT cell frequencies were associated with death in severe avian H7N9 IAV disease.

Activation has also been observed by others in hepatitis B, hepatitis C and Zika infections, whilst reductions in both MAIT cell activation and function in the lungs following IAV infection of mice.

**Results**

MAIT cells accumulate and are activated during influenza.

First, we sought to determine whether MAIT cells were activated in vivo during influenza virus infection. For these studies, C57BL/6 (wild-type; WT) mice received an experimental challenge with 100 plaque forming units (PFU) of the mouse-adapted influenza virus strain A/Puerto Rico/8/34/1934 (PR8, H1N1), which causes severe pneumonia in mice, characterised by parenchymal necrosis and infiltrates of macrophages, lymphocytes and neutrophils. We observed rapid accumulation of pulmonary MAIT cells (defined as TCR\( \beta \)-CD45.2+CD19- MR1-5-OP-RU tetramer+ cells, see Supplementary Fig. 2), which peaked around 5 days post-infection (dpi) (Fig. 1a, mean 2.6-fold increase from baseline, Kruskal–Wallis with post hoc Dunn’s \( P < 0.0001, n = 20 \), returning to baseline by day 7 (Fig. 1a, b)). By comparison, peak frequencies of non-MAIT (‘conventional’) CD8+ cells occurred later at day 7 post-infection. The proportional increase in MAIT cells was significantly higher than that of CD4+ or CD8+ cells early during infection at day 5 post-infection (\( P < 0.001 \)), though not by day 7 at which point antigen-specific responses will become dominant over other innate-like cells. In addition to MAIT cell recruitment, IAV infection induced MAIT cell activation, as indicated by increased expression of CD25, CD69 and Granzyme B (Fig. 1c–h).

Expression of CD25 and CD69 peaked at day 5 post-infection and was significantly higher in MAIT cells than conventional CD4+ or CD8+ T cells, persisting until day 13 post-infection. By contrast, CD25 expression on conventional CD4+ T cells peaked at 7 dpi, whilst CD69 continued to increase over the course of the experiment and the highest levels were detected on CD4+ and CD8+ T cells at 13 dpi. Together, these results indicate that MAIT cells accumulate and are activated early in the airways following IAV infection of mice.

**Accumulation and activation is modulated via cytokines.** We have previously demonstrated that MAIT cells can be activated by viruses independently of MR1, via IL-18 in synergy with IL-12, IL-15 or IFN-\( \alpha /\beta \). We therefore investigated the role of these cytokines in the accumulation and activation of MAIT cells in the lungs following IAV infection, using mouse strains deficient in particular cytokines or cytokine receptors. As an additional control for MR1-mediated activation, intravenous injection was used to adoptively transfer C57BL/6 pulmonary MAIT cells into MR1−/− mice, which otherwise lack MAIT cells, as previously described. Compared with WT C57BL/6 mice, the magnitude of influenza virus-induced pulmonary MAIT cell accumulation was unaffected by deficiency in IL-12, IL-15, IFN-\( \alpha /\beta \) receptor (IFNAR) or MR1 (Fig. 2a, mean 2.1–2.9-fold increases above baseline at 5 dpi), but was significantly impaired (Kruskal–Wallis with post hoc Dunn’s \( P < 0.05 \)) by deficiency in IL-18 (mean 1.3-fold increase) (Fig. 2a).

By contrast, MAIT cell activation (as determined by measurement of CD25 expression) was significantly impaired by deficiency of IL-15, -18 or IFNAR (showing 69%, 70% and 67% reductions in the proportion of MAIT cells expressing CD25 respectively, \( P < 0.05 \) for each) and most dramatically by deficiency of IL-12 (90% decrease, \( P < 0.0001 \)) (Fig. 2b). Differences in CD69 expression were not statistically significant (Fig. 2c).

These results support the concept that in vivo, MR1-independent MAIT cell activation is driven by virus-induced pro-inflammatory cytokines, with a dominant role for IL-18, as previously suggested by in vitro studies.

**MAIT cell-deficient mice show enhanced mortality.** Given that MAIT cells are activated and accumulate in the lungs following influenza virus infection, we sought to determine whether they might play a protective role or, conversely, enhance the inflammatory cytokine milieu and contribute to exacerbated immune
pathology. We therefore examined weight loss and survival following influenza virus infection of WT C57BL/6 mice and MR1−/− mice, which have an absolute deficiency of MAIT cells. Following challenge with 100 PFU of PR8 virus, MR1−/− mice exhibited greater body weight loss in comparison with WT mice. Importantly, the body weight loss and mortality in MR1−/− mice were ameliorated by i.v. adoptive transfer of pulmonary MAIT cells at 1 week prior to influenza virus infection.

We did not observe significant differences in viral load in lung homogenates prepared from PR8-infected C57BL/6 and MR1−/− mice at days 3 and 5 post-infection. However, MR1−/− mice had reduced numbers of SigF+CD11bintCD64+CD11c+ alveolar macrophages at 3 dpi (Fig. 3d, Kruskal–Wallis with post hoc Dunn’s P < 0.05) and reduced pulmonary T cell numbers by 5 dpi (Fig. 3e, P < 0.001). The absolute numbers of neutrophils, eosinophils, dendritic cells, NK cells and γδ T cells across the time points post PR8 infection were not statistically significant (Supplementary Fig. 3). Using tetramers for MR1 or to immunodominant epitopes from the nucleoprotein or polymerase acidic protein of influenza virus, we tracked MAIT cells and conventional antigen-specific αβ T cell accumulation during PR8 infection. In MR1−/− mice, we observed a tendency towards decreased accumulation of antigen-specific T cells, at day 9 in splenocytes, and for nucleoprotein at day 7 in the lymph nodes (Supplementary Fig. 4) suggesting MAIT cells may enhance the development of an effective antigen-specific adaptive immune response. Total protein concentrations in cell-free BAL supernatants, a sensitive marker of pulmonary epithelial damage,
were also increased in MR1−/− mice at 5 dpi (Mann–Whitney P < 0.05, Fig. 3). These data indicate that early accumulation of MAIT cells at the site of infection protects against influenza-induced morbidity and mortality associated with excessive lung damage and recruitment of inflammatory cells, but not with viral killing.

As the mouse-adapted PR8 strain is highly virulent in mice, we performed additional experiments using X-31 (H3N2, a reassortant virus bearing the hemagglutinin and neuraminidase genes from A/H1N1/1918 and the remaining six genes from PR8), a strain that shows moderate virulence in mice. After intranasal infection of WT or MR1−/− mice with 5000 PFU of X-31, we did not observe significant differences in survival or in titres of infectious virus detected in the lungs (Supplementary Fig. 5a–c), although we did observe a similar virus-induced pulmonary accumulation of MAIT cells as well as virus-induced

**Fig. 2** MAIT cell accumulation and activation are modulated through cytokines, independent of MR1. Accumulation and activation of MAIT cells in wild type (WT) C57BL/6 mice or mice deficient in IL-12, IL-18, IFNαR or MR1 5 days after challenge with 100 plaque-forming units (PFU) PR8 virus. Mice deficient in MR1 had received adoptive transfer of 1.8 × 10⁵ pulmonary MAIT cells 2 weeks earlier, from WT mice previously infected with 10⁶ CFU S. Typhimurium BRD509 for 7 days to expand the MAIT cell population. Cells were transferred 1 week prior to infection with 100 PFU PR8 virus. WT (n = 7) and MR1−/− (n = 9) data are representative of four experiments with similar results. Adoptive transfer (n = 6), performed once, used 1 × 10⁶ pulmonary MAIT cells from WT mice previously infected with 10⁶ CFU S. Typhimurium BRD509 for 7 days to expand the MAIT cell population. Cells were transferred 1 week prior to influenza virus infection. Graphs show mean weights ± SEM for surviving mice, with individual animals at 3, 5 and 7 dpi. Data are combined from three experiments with similar results. Groups compared with WT by Mann-Whitney U tests. *P < 0.05, **P < 0.01, ***P < 0.001

**Fig. 3** MR1−/− mice show enhanced weight loss and mortality in response to severe influenza. a Body weight loss expressed as a percentage after infection with 100 PFU PR8 virus. WT (n = 7) and MR1−/− data (n = 9) are representative of four experiments with similar results. Adoptive transfer (n = 6), performed once, used 1 × 10⁵ pulmonary MAIT cells from WT mice previously infected with 10⁶ CFU S. Typhimurium BRD509 for 7 days to expand the MAIT cell population. Cells were transferred 1 week prior to influenza virus infection. Graphs show mean weights ± SEM for surviving mice, with individual animals at 3, 5 and 7 dpi. Data are combined from three experiments with similar results. Groups compared with WT by Mann-Whitney U tests. *P < 0.05, **P < 0.01, ***P < 0.001

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MAIT cell activation (2.6-fold increase at 5 dpi, Supplementary Fig. 5d–g). Furthermore, following infection with either PR8 or X-31, we observed reductions in key innate inflammatory cell cytokines in the lungs of MR1−/− compared with wild-type C57BL/6 mice. In particular, in MR1−/− mice we detected significantly lower amounts of monocyte chemotactic protein 1 (MCP-1/CCL2) and IL-6 following infection with 100 PFU of PR8, and of RANTES/CCL5 following 5000 PFU of X-31 with similar trends observed with both virus strains for each of the inflammatory mediators detected (Supplementary Fig. 6). Likewise, there were lower levels of interferon-γ in bronchoalveolar lavage fluid at day 5 post-infection with 100 PFU of PR8 in MR1−/− mice (3.2-fold difference in geometric mean, t-test on log-transformed data, P = 0.044) (Supplementary Fig. 7).

Together, these data indicate that MAIT cells play a protective role during severe influenza virus infection, enhancing pulmonary T cell accumulation and innate inflammatory cytokine production, and reducing weight loss, mortality and pulmonary epithelial damage.

MAIT cell transfer delays mortality in immunodeficient mice.

To further explore the protective role of MAIT cells during influenza infection, we studied the impact of adoptively-transferred MAIT cells into immunodeficient Rag2−/−γC−/− mice lacking T cell-, B cell- and NK cell-mediated immunity. As previously described14,29, ~0.3 × 10⁶ pulmonary MAIT cells were adoptively transferred into Rag2−/−γC−/− mice. Low numbers of residual contaminating conventional T cells from the donor mice can rapidly expand in these recipient mice. Therefore, residual contaminating conventional T cells were depleted in recipients with anti-CD4 and anti-CD8 mAbs, and the animals rested for 1–2 weeks to allow expansion of the MAIT cell population prior to challenge with PR8 or X-31 strains (Fig. 4a). Adoptive transfer of MAIT cells reduced weight loss (Fig. 4b) and significantly prolonged survival after PR8 infection (Fig. 4c, log-rank Mantel–Cox P < 0.05). Furthermore, when we directly compared the protective effect of MAIT, CD8 and NK cells in the same model, MAIT cell transfer was associated with prolonged survival compared with CD8 transfer (Bonferroni-corrected log-rank Mantel–Cox P = 0.009), but was not superior to NK cell transfer (P = 0.8, Supplementary Fig. 8). Moreover, in this immunocompromised Rag2−/−γC−/− mouse model adoptive transfer of MAIT cells now also provided protection following challenge with the less virulent X-31 strain (Fig. 4d, e, log-rank Mantel–Cox P = 0.02).

Human MAIT cells express IFN-γ in response to stimulation of TLR-8 with single-stranded RNA20, and also that human viral activation of MAIT cells can suppress replication of hepatitis C virus via IFN-γ in vitro31, we hypothesised that IFN-γ expression by MAIT cells could contribute to their protective role following influenza virus infection. To address whether IFN-γ expression by MAIT cells confers protection against IAV in vivo, MAIT cells from wild-type C57BL/6 and from IFN-γ−/− donor mice were transferred into Rag2−/−γC−/− mice. As expected, the protective effect of adoptively-transferred MAIT cells was abrogated when the transferred MAIT cells were deficient in IFN-γ (Fig. 4d, e), suggesting that the antiviral effects provided by MAIT cells were mediated, at least in part, by IFN-γ.

Discussion

We have shown in vivo that MAIT cells accumulate in the lungs, are activated and contribute to protection against morbidity and mortality during influenza virus infection in mice. These findings are consistent with our previous human in vitro observations in which we demonstrated MR1-independent MAIT cell activation in response to exogenous cytokines20,21 or to IAV21. In those experiments, we co-incubated peripheral blood mononuclear cells (PBMCs) or human CD8 T cells with virus-infected monocytes, macrophages or dendritic cells and observed strong, dose-dependent MAIT cell activation with upregulation of CD69 and IFN-γ and Granzyme B21. We also observed virus-induced upregulation of CD69, Granzyme B and IFN-γ on MAIT cells in separate experiments using co-culture of PBMC with IAV infection of a human lung epithelial cell line (A549)22. Here, we observed strong CD25 and CD69 upregulation by MAIT cells in the lungs of IAV-infected mice, which occurred earlier and to a greater extent than in non-MAIT CD3+ T cells, and was associated with Granzyme B upregulation, suggesting that these cells are licensed for cytotoxicity early in influenza virus infection.

In the previous in vitro studies, virus-induced responses were unaffected by anti-MRI blocking antibody21,22, but rather were driven by cytokines. Indeed, this would be consistent with the MAIT cell accumulation (Fig. 2a) and their protective effects observed after adoptively transferring MAIT cells into mice with deletion of MR1 (Fig. 3b). Stimulation with IL-12 and IL-18 is known to induce IFN-γ from CD161++ CD8+ T cells, of which MAIT cells are the dominant subset in humans20. In both the lung epithelial cell22 and the professional antigen presenting cell21 co-culture systems, we found IL-18 to be the dominant activating cytokine in influenza virus infection, acting synergistically with IL-12 or IL-15 or type I interferons21. Again, we confirmed these observations in vivo using mice with genetic deletions of these cytokines or their receptors, and found that MAIT cell accumulation and CD69 upregulation were significantly impaired only in the IL-18−/− mice (Fig. 2a, c). Interestingly, CD25 activation was affected to the greatest extent by IL-12 deletion (Fig. 2b), likely because an important general biological function of IL-12 is to induce T cell upregulation of CD2521.

Our previous clinical human influenza infection studies showed activation of peripheral blood MAIT cells, measured by Granzyme B upregulation21 and a decrease in peripheral blood MAIT cell frequencies, which correlated with disease severity21 and with fatal outcomes22. In these studies, it was not possible to determine causal associations nor examine lung tissue. Our murine data recapitulated these findings and revealed activated MAIT cells at the site of infection. In human studies, there are wide inter-individual variations in baseline frequencies of peripheral blood MAIT cells4. However, overall the observed clinical association between reduced MAIT cell frequencies and influenza mortality would be consistent with the increased mortality we observed here in MR1−/− mice.

MAIT cells are not unique in their capacity to respond to virus-induced cytokines. Virus-induced bystander activation was first described in conventional CD8+ T cells in mice32 but is overall a rare event in these cells33. However, the high IL-18R expression and IL-12/18 responsiveness appear to be common to a range of innate-like lymphocytes expressing PLZF34, which in humans include iNKT, γδ T, and NK cells33. Indeed, protective roles for iNKT cells have been shown in murine IAV infection36–38. Nonetheless, whilst iNKT cells are relatively abundant in mice, they are quite rare in humans, constituting approximately 0.1% of peripheral blood T cells3,35,39. In contrast, human MAIT cells are much more abundant comprising 1–10% of blood3 and pulmonary4 T cells. Furthermore, at early stages the absolute numbers of these cells in the lungs will markedly exceed the numbers of conventional antigen-specific T cells responding to cognate viral antigens34. Furthermore, as they display a significantly higher TCR-independent upregulation of IFN-γ than conventional T cells22, MAIT cells may have a considerable role in early antiviral protection in humans. Nevertheless, despite much lower relative frequencies of MAIT cells in mice compared
with humans, it is striking that we could observe a protective effect of MAIT cells.

Although MAIT cells protected against influenza virus-induced weight loss and mortality, we did not detect a significant difference in viral titres at the time of peak viral load (i.e. 3–5 dpi). This is not unexpected, as other investigators have also observed significant differences in immunopathology without changes in IAV load. Furthermore, whilst the cytokine responsiveness of MAIT cells suggests the potential for a possible deleterious role in which they could contribute to the ‘cytokine storm’ effect observed in severe influenza, our data from both C57BL/6 and RAG2−/− γC−/− strains showed no evidence that MAIT cells contributed to enhanced immunopathology. In fact, the opposite effect was seen. This is a critical observation because, although it is known that MAIT cells home to sites of inflammation and are activated by IL-18/-15/-12 to produce IFN-γ, and that IFN-γ can be antiviral. Only a direct in vivo challenge experiment is able to directly answer the question as to whether, in a complex host–pathogen interaction within an intact organism, the protective effects of these mechanisms outweigh the detrimental immunopathological consequences.

The importance of MAIT cells to immune protection in humans may be most apparent when MAIT cell frequencies are suppressed. Of particular relevance to respiratory viral infections, therapeutic use of inhaled and oral corticosteroids causes marked reductions in airway MAIT cell frequencies in asthma and chronic obstructive pulmonary disease, and thus may contribute to the increased risk of influenza hospitalisation in these conditions.

Influenza virus is a major human respiratory pathogen, which causes seasonal epidemics resulting in 300–650,000 deaths...
annually.46 IAV can also cause pandemic spread when novel strains emerge.45 Although the most effective method for prevention and control of influenza is vaccination, current vaccines show limited efficacy and little heterologous protection between strains, and so require annual reformulation. The TCR-independent nature of the antiviral MAIT cell response therefore offers significant therapeutic potential in at least two ways. Firstly, strategies to target MAIT cell activation, for instance by incorporating MAIT cell ligands and TLR agonists in the formulation of novel adjuvants, could be used to enhance the efficacy of future influenza vaccines. Secondly, we have shown that MAIT cell frequencies can be rapidly ‘boosted’ through mucosal administration of simple synthetic MAIT cell ligands with TLR agonists,44,47 which may prove effective in a number of scenarios including short-term prophylactic vaccination during an epidemic. Such a strategy may also prove beneficial in protecting groups known to show impairments in MAIT cell immunity—such as the elderly or those receiving therapeutic corticosteroids—even acutely during the early stages of infection.

In summary, we have shown for the first time in vivo evidence of a significant contribution of MAIT cells to protective immunity against a major human viral pathogen. As the cytokine-mediated activation of MAIT cells is a mechanism likely to be common to a number of other acute and chronic viral infections, as has already been shown in dengue virus, hepatitis C virus in clinical studies,21,28 these findings from an influenza model are likely to be widely applicable to a range of respiratory and systemic viral diseases.

Methods

Animal models. Mice were bred and housed in the Biological Research Facility of the Peter Doherty Institute (Melbourne, Victoria, Australia). MR1−/− mice were generated by breeding Vα19Gαc−/− MR1−/− mice26 from (Susan Gilliland, Washington University, St. Louis School of Medicine, St. Louis, MO) with C57BL/6 mice and inter-crossing of F1 mice. The genotype was determined by tail DNA PCR at the MR1 locus using the following primers:26 Fwd: AGC TGA AGT CTT TCA AGA TCG; Rev (wild type): ACA GTC ACA CAG GAG TGG TGC; Rev (knockout): GAT TCT GTG AGC CCT GTG TGC. IFN-γ knockout mice (B6.129S7-Ifngm1J/J)48 are commercially available (Jackson Laboratory). Male mice aged 6–12 weeks, matched for age, sex and weight, without randomisation or blinding, were used in experiments, after approval by, and in accordance with, the requirements of the University of Melbourne Animal Ethics Committee (1513661).

Intranasal virus infection. Intranasal (In.) inoculation with 50–1 × 10^4 PFU of X-31 (H3N2; A/Hong Kong/334/1934 IAV, or with S. Typhimurium BRD509 (10^6 colony forming units (CFU)) in 50 μL PBS containing Trypsin (Worthington Biochemical, NJ, USA), as described.49 Plates were incubated at 37 °C, 5% CO2 for 3 days before plaques were counted.

Determination of viral load counts in infected lungs. Viral load was determined by counting PFU in MDCK monolayers infected with lung homogenates, at varying dilutions for 45 min at 37 °C. 5% CO2, before the addition of an Agarose/ L15 or MEM overlay containing Trypsin (Worthington Biochemical, NJ, USA), as described.49 Plates were incubated at 37 °C, 5% CO2 for 3 days before plaques were counted.

Adoptive transfer. As MAIT cell numbers are low in naive C57BL/6 mice, prior to adoptive transfer experiments MAIT cell populations were expanded by intranasal inoculation with 1 × 10^6 CFU of S. Typhimurium BRD509 in 10 μL PBS as described.49 After 7 days, mice were sacrificed, single-cell suspensions prepared and live CD^3^- CD^4^5^- MR1^-/- OP-RU tetramer+ cells sorted using a BD FACS Aria III. Simultaneously, from these single cell suspensions, live CD^3^ - CD^4^5^- CD^8^5^- MR1^-/- OP-RU tetramer+ were sorted for CD^8^+ T cell adoptive transfer. For the transfer of NK cells, prior to cell sorting, single cell suspensions from naive WT spleens were subjected to magnetic bead-based antibody depletion with anti-CD11b, anti-CD4, anti-CD8 and anti-B220 (reagents kindly provided by Professor Alex Kallies). Live NK1.1^-/- CD3^- CD4^- CD8^- B220^- CD11b^- CD11c- cells were sorted using 5 × 10^6 pulmonary MAIT cells were injected into the tail of recipient Rag2^-/- CD4^-/- CD8^- mice which then received 0.1 mg each of anti-CD4 (24G2) and anti-CD8 (53.762) mAb i.p. on days 2 and 5 to control residual donor-derived conventional T cells. Mice were rested for 2 weeks post transfer to allow full expansion of the MAIT cell population prior to infectious challenge. In a separate set of experiments, 1 × 10^5 pulmonary MAIT or CD8+ cells were splenic NK cells were transferred to recipient Rag2^-/- CD4^-/- CD8^- mice as described above. Prior to infectious challenge, venous tail blood was stained to confirm the presence of the transferred cell subsets.

Reagents. MR1^-/- OP-RU-SA BV421 tetramers were generated as described previously49 (now available from the NIH core tetramer facility) and in multiple groups. Survival curves were compared using log-rank (Mantel-Cox) tests (two groups) or the Gehan-Breslow-Wilcoxon method for multiple groups. Data were acquired using FACSComp software (version 7.0, La Jolla, CA). Comparisons were performed using the Prism GraphPad software (version 7.0, La Jolla, CA). A comparison of the control and treatment groups over time was performed using the non-parametric equivalent of analysis of variance (Kruskal-Wallis test) and the Dunn test post analysis. The CBA ex set (BD Bioscience) was used as per manufacturer’s instructions: Live/Dead Fixable Aqua, ThermoFisher, Waltham, MA, USA) and ZombieYellow (423104, Biolegend).

Statistical analysis. Statistical tests were performed using the Prism GraphPad software (version 7.0, La Jolla, CA). Comparisons were performed using Mann–Whitney tests (two groups) with Bonferroni corrections for two-tailed comparisons, or with Kruskal–Wallis one-way ANOVA with post hoc Dunn’s tests (multiple groups). Survival curves were compared using log-rank (Mantel–Cox) tests (two groups) or the Gehan–Breslow–Wilcoxon method for multiple groups. Variance was similar in each case between groups being compared. Sample sizes were predicted experience of at least 20 PBS X-31 strains. All statistical tests are two-sided. Flow cytometric analysis was performed with FlowJo10 software (Ashland, OR).
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Author contributions
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