Published in final edited form as:

Immunol Cell Biol. 2020 October ; 98(9): 770–781. doi:10.1111/imcb.12370.

The MAIT TCR β chain contributes to discrimination of microbial ligand

Gitanjali A. Narayanan¹, James E. McLaren², Erin W. Meermeier¹, Kristin Ladell², Gwendolyn M. Swarbrick^{1,3}, David A. Price^{2,4}, Jessica G. Tran³, Aneta H. Worley³, Todd Vogt³, Emily B. Wong^{5,6,7}, David M. Lewinsohn^{1,3}

¹Department of Pulmonary and Critical Care Medicine, Oregon Health & Science University, Portland, OR 97239, USA

²Division of Infection and Immunity, Cardiff University School of Medicine, Heath Park, Cardiff CF14 4XN, Wales, UK

³VA Portland Health Care Center, Portland, OR 97239, USA

⁴Systems Immunity Research Institute, Cardiff University School of Medicine, Heath Park, Cardiff CF14 4XN, Wales, UK

⁵Africa Health Research Institute, Durban, South Africa

⁶Division of Infectious Diseases, Massachusetts General Hospital, Boston, MA, USA

⁷Harvard Medical School, Boston, MA, USA

Abstract

Mucosal Associated Invariant T (MAIT) cells are key players in the immune response against microbial infection. The MAIT T cell receptor (TCR) recognizes a diverse array of microbial ligands and recent reports have highlighted the variability in the MAIT TCR that could further contribute to discrimination of ligand. The MAIT TCR CDR3 β sequence displays a high level of diversity across individuals and clonotype usage appears to be dependent on antigenic exposure. To address the relationship between the MAIT TCR and microbial ligand, we utilized a previously defined panel of MAIT clones that demonstrated variability in responses against differing microbial infections. Sequencing of these clones revealed four pairs, each with shared CDR3 α and differing CDR3 β sequences. These pairs demonstrated varied responses against microbially infected dendritic cells, as well as to 5-(2-oxopropylideneamino)-6-D-ribitylaminouracil, a ligand abundant in *Salmonella enterica* serovar Typhimurium, suggesting that the CDR3 β contributes to differences in ligand discrimination. Taken together, these results highlight a key role for the MAIT CDR3 β region in distinguishing between MR1-bound antigen.

Graphical Abstract

Correspondence: David M. Lewinsohn, Pulmonary and Critical Care Medicine, OHSU, 3181 SW Sam Jackson Park Rd, Mail Code VA R&D 11, Portland, OR 97239, USA. Tel: 503-273-5138. Fax: 503-402-2816 lewinsod@ohsu.edu.

CONFLICT OF INTEREST: The authors declare no conflict of interest

The contents of this manuscript do not represent the views of the U.S. Department of Veterans Affairs or the United States Government.

Mucosal Associated Invariant T (MAIT) cells can recognize and respond to a wide range of microbial infection. The MAIT T cell receptor is known to be increasingly diverse and bind a wide range of microbial ligand. We show that the MAIT T cell receptor beta chain can discriminate between microbial infection as well as ligand.

Keywords

Antimicrobial responses; bacterial host response; T-cell receptor

INTRODUCTION

Mucosal-associated invariant T (MAIT) cells are key players in the immune response against microbial infection^{1, 2}. In humans, MAIT cells represent 1-10% of all circulating T cells and are activated by microbial antigen presented by MR1 on the surface of infected cells^{3, 4}. MAIT cells are highly abundant at mucosal sites and exit the thymus with innate effector functional capacity and are therefore thought to play an role in the containment of microbial infection^{3, 5}. Mice lacking functional MAIT cells or MR1 have been shown to display decreased bacterial clearance and impaired recruitment of the adaptive immune response, further supporting the proposed role of MAIT cells in controlling microbial infection⁶⁻¹¹.

Human MAIT cells have been defined by the expression of a semi-invariant T cell receptor (TCR) comprising a *TRAV1-2*⁺ TCR α (TRA) chain, encoding a limited repertoire of *TRAJ* genes, paired to a TCR β (TRB) chain with varied *TRBV* usage^{4, 12}. The first-described activating MR1-restricted ligands were antigens produced from the interactions of biosynthetic intermediates in the riboflavin synthesis pathway with other small metabolites¹³⁻¹⁵. These compounds are produced by microbes that stimulate MAIT cell responses, thereby introducing a mechanism of self vs. non-self-recognition by MAIT cells.

Recent reports have demonstrated that the TCR repertoire as well as diversity ligands capable of stimulating T cells by MR1-mediated antigen presentation broader than previously thought. This repertoire includes ligands generated from non-riboflavin synthesizing microbes, such as *Streptococcus pyogenes*, which activate *TRAV1-2*- T cells through antigen presentation by MR1, as well as ligands derived from cultured mouse tumor cells and human monocytic cell lines¹⁶⁻¹⁸. *In silico* binding assays and functional studies utilized to explore the landscape of potential MAIT cell reactive antigens showed that compounds lacking the ribityl tail of canonical MR1-restricted ligands, including pharmacologic agents, such as diclofenac, could stimulate MAIT cells¹⁹. Recently, metabolomic analyses revealed the identity of shared or distinct non-riboflavin derived antigens from *Escherichia coli* (*E. coli*) and *Mycobacterium smegmatis* (*M. smegmatis*) to activate MAIT cells, as well as non-riboflavin derived activating ligands identified in *M. smegmatis*²⁰.

The relative contribution of MAIT cell *TRA* and *TRB* chains in ligand recognition is incompletely understood. Biochemical analysis suggested a critical role for the CDR3 α loop in antigen recognition, with Tyr95 α predicted to mediate hydrogen bond contacts with the ribityl chain of riboflavin-derived ligands, more recently, described to form an ' interaction

triad' with Tyr152 from MR1 and 5-OP-RU^{15, 21, 22}. While mutagenesis of single residues within the MAIT CDR3 β loop had no discernable effect on ligand recognition, exchange of the entire MAIT CDR3 β region for a non-MAIT cell derived CDR3 β sequence ablated antigen recognition²³. However, Lopez-Sagaseta and colleagues used recombinant humanized forms of bovine MR1 exposed to ligands present in *E. coli* culture supernatant to demonstrate that the MAIT CDR3 β loop is important for contact with MR1-bound antigens²⁴. Further work shows that hypervariability in the MAIT CDR3 β contributes to fine-tuning ligand-TCR interactions, and the flexibility demonstrated in the CDR3 β suggests a further fine-tuning of TCR-ligand interactions²³⁻²⁵. While these data suggest that both *TRA* and *TRB* chains contribute to ligand recognition, the relative contribution of individual TCR chains to the recognition of microbial infections remains to be elucidated.

Increasing evidence suggests that the MAIT TCR repertoire is shaped by exposure to microbial ligands. Analyses of TCR repertoire patterns in circulating, microbially reactive MAIT cells demonstrated diversity in the CDR3 β chain usage²⁶. For a given individual, CDR3 α usage was limited and could be linked to a specific microbe. Furthermore, Dias et al have reported that the *TRBV* usage in MAIT cell-specific TCRs contributes to responses to MR1-bound antigen and the magnitude of this usage appears to be microbe-dependent²⁷. In contrast, Lepore and colleagues found that the CDR3 β region is of limited diversity and is shared across individuals²⁸. More recently, it has been shown experimentally that vaccination with *Salmonella enterica* serovar Paratyphi A resulted in the clonal expansion of MAIT cells, leading to a CDR3 β repertoire that is both biased and oligoclonal²⁹. This raises the intriguing possibility of the MAIT TCR repertoire in an individual adapts to microbial exposure.

Here, we show that a number of MAIT cell clones sharing identical CDR3 α regions with differing CDR3 β regions display a diverse response profile to microbially infected dendritic cells (DCs). Selective recognition of distinct microbes and 5-OP-RU, a ligand found to be abundant in *Salmonella enterica* serovar Typhimurium (*S. Typhimurium*) was clonotypically linked to the individual CDR3 β region in each responding clone. Taken together, these results support the growing body of evidence that the MAIT CDR3 β chain is contributing to the discrimination of bacterial ligands.

RESULTS

MAIT cell clones display diverse responses to microbially infected DCs

We have previously utilized limiting dilution analysis to generate a panel of *Mycobacterium tuberculosis* (Mtb) reactive CD8+ T cell clones from PBMC of healthy or Mtb-infected individuals. These clones all are *TRAV1-2*⁺ and recognize microbial antigen bound to MR1, as measured using a blocking antibody to MR1. We have previously found that these clones were not dependent on HLA-Class Ia or CD1². Additionally, these clones did not produce IFN- γ in response to TLR stimulation. Furthermore, neither TLR blockade nor blockade of NK receptors impeded cytokine production in response to infected DCs². From this original work, 120 MAIT cell clones were subsequently tested for production of IFN- γ in response to DCs infected with Mtb, *Mycobacterium smegmatis* (*M. smegmatis*), *Mycobacterium marinum* (*M. marinum*), or *Escherichia coli* (*E. coli*). As shown in Figure

1a, MAIT cell responses were quantitatively diverse. To understand whether or not these responses could be grouped functionally, linear regression analysis was performed and correlation between microbial responses was analyzed. As demonstrated in Fig 1b, responses to *M. smegmatis* and *E. coli* were similar (Pearson's $r = 0.74$, $n = 86$), while Mtb and *M. marinum* were highly correlated (Pearson's $r = 0.74$, $n = 42$). These data suggest that *M. smegmatis* and *E. coli* share a similar ligand repertoire, as do Mtb and *M. marinum*.

MAIT TCR sequencing reveals clones sharing identical CDR3 α regions but distinct CDR3 β sequences

To establish the relationship between TCR clonotype and microbial discrimination, we sought to characterize the clonotypic repertoire in our panel of MAIT cell clones. *TRAV1-2*⁺ CD8⁺ MAIT cell clones (Figure 2a) were evaluated for MR1-dependent IFN- γ production in response to *M. smegmatis* infected A549 cells (Figure 2b). In addition, we included a panel of MAIT cell clones isolated from bronchoalveolar lavage of Mtb-infected individuals ³⁰.

The TCR sequence of each validated MAIT clone was determined using an unbiased template switch anchored RT-PCR approach, as previously described ³¹. The majority of sequenced MAIT cell clones expressed *TRAV1-2/TRAJ33* encoded TRA chains that were paired to *TRBV6* or *TRBV20-1* TRB chains, although exceptions were observed where other *TRBV* genes were encoded (e.g. *TRBV3-1*, *TRBV4-2*, *TRBV19*; Supplementary Table 1). All sequences were analyzed for similarity to previously reported MAIT CDR3 α sequences using an algorithm based on the method described by Shen et al, 'MAIT Match', where reported MAIT CDR3 α sequences are analyzed for similarity to published sequences ³². Based on our prior analyses of MAIT CDR3 α chains using this tool, we chose a MAIT Match score of 0.95 as a conservative threshold to define MAIT consistent TCRs. Using this analysis, we show that the majority of the clones were verified to display a high degree of similarity to previously described MAIT cell specific TCRs ³⁰ (Supplementary Table 1). Despite evidential publicity in the CDR3 α region of sequenced MAIT cell clones, it was apparent that there was no clonotypic sharing of CDR3 β regions across donors. Of the 35 clones sequenced, we identified four "pairs" of MAIT cell clones that shared an identical CDR3 α sequence with differing CDR3 β sequences (Table 1). To our surprise, one pair arose from the same donor (e.g. D0033A2 and D0033A10) and exhibited distinct CDR3 β sequences despite both utilizing *TRBV3-1* gene usage. This indicated that there is no clonotypic sharing in CDR3 β region of MAIT TCRs across individuals.

Paired MAIT clones differentially recognize microbially infected dendritic cells

While the role of the CDR3 β in recognition of peptide ligand presented on classical MHC is well defined, it is not clear the extent to which the MAIT TCR β chain contributes to recognition of microbial antigens. Consequently, we tested each paired MAIT for responsiveness to dendritic cells infected with *S. Typhimurium*, *Candida albicans*, (*C. albicans*) or *M. bovis* BCG (BCG) (Figure 3). These microbes were chosen to reflect ligands that might be derived from diverse microbial species known to stimulate MAIT cells through their TCRs

While 427D8-2 responded to *S. Typhimurium*, *C. albicans*, and BCG at magnitudes comparable to the MAIT control clone, 426G11, the magnitude of 450B9 responses to these infections was 2-fold weaker for BCG ($P = 0.0008$), and nearly 3-fold weaker for both *S. Typhimurium* ($P < 0.0001$) and *C. albicans* ($P < 0.0001$). This suggests while 450B9 appears to have diminished functional avidity, it seems to be independent of the TCR β .

In contrast, the clone pair 403A9 and D0033A6 yielded a very different result. Specifically, responses by 403A9 and D0033A6 were similar in magnitude to BCG infection. However, while 403A9 responded robustly to *S. Typhimurium*, the D0033A6 response was 2-fold weaker ($P = 0.016$). The D0033A6 response to *C. albicans* was similar to that of 403A9. These results indicate that the differences in the CDR3 β between D0033A6 and 403A9 could contribute to the recognition of *S. Typhimurium*-derived antigens, but not to antigens derived from BCG or *C. albicans*.

Responses of 450A9 and 571F3-2 to *S. Typhimurium* and *C. albicans* were similar in magnitude, but 450A9 responses to BCG were significantly weaker than 571F3-2 ($P = 0.039$). The clone pair D0033A2 and D0033A10, however, displayed no differences in magnitude when responding to all three infections, suggesting that the differences in the TRB chain did not confer specificity in recognizing antigen.

Prior reports have also demonstrated that non-MR1 mediated means of activation, such as TLR signaling or cytokine stimulation, can also promote MAIT cell production of IFN- γ ³³⁻³⁵. Therefore, we sought to determine whether differences in MAIT cell CDR3 β region confer selectivity against a MR1-restricted antigen synthesized by *S. Typhimurium*, and is highly abundant in that microbe, 5-(2-oxopropylideneamino)-6-D-ribitylaminouracil (5-OP-RU) in the absence of any MR1-independent signaling¹⁴. Accordingly, we utilized a plate-bound MR1/5-OP-RU tetramer ELISpot (tetraSPOT) assay, which we have previously demonstrated will activate MAIT cell clones²⁰, to evaluate MR1/5-OP-RU specific responses in each pair of MAIT cell clones (Figure 4). In this manner, we were able to measure MAIT production of cytokine directly as a response of TCR engagement with MR1-bound antigen. As a result, we could exclude APC dependent factors such as co-stimulation or cytokine production.

Here, the MR1/5-OP-RU tetramer was plated over a range of concentrations to assess the functional avidity of each clone, expressed by EC₅₀, which has been previously established³⁶. All MAIT cell clones responded to the plate bound MR1/5-OP-RU tetramer, and each response profile and avidity measurement largely mirrored that observed in functional assays with *S. Typhimurium* infected DCs (Figure 4a). Most notably, the 403A9 clone had an EC₅₀ that was 4-fold higher than D0033A6. In contrast, the responses between the other paired clones were similar, both in magnitude and in EC₅₀, again corresponding with the responses of MAIT clones to *S. Typhimurium*. The responses of the paired clones to a MR1/6FP control tetramer was not detectable (Figure 4a).

Finally, as our initial screen had relied on detection of the MAIT TCR by staining for *TRAV1-2*, we stained the clones with the MR1/5-OP-RU tetramer as a surrogate for TCR avidity (Figure 4b). Intriguingly, while all clones stained with the MR1/5-OP-RU tetramer,

D0033A6 displayed a much lower affinity of staining compared to 403A9, mirroring the results seen in the plate-bound tetramer assay. The other paired clones displayed similar staining intensity with the MR1/5-OP-RU tetramer, correlating with the plate-bound tetramer assay. Additionally, the paired clones displayed similar intensity of staining with CD8 and CD3 (Figure 4c and 4d). Taken together, these findings suggest that the MAIT CDR3 β loop is an important component for ligand discrimination during microbial infections, notably the ligand 5-OP-RU.

DISCUSSION

To explore the relationship of ligand discrimination and TCR usage, we sequenced TCRs from a panel of *TRAV1-2*⁺ MAIT cell clones. The diversity in the CDR3 α and CDR3 β chains was consistent with prior reports^{17, 26, 28}. Through the identification of clone pairs that shared identical CDR3 α loops but possessed unique CDR3 β regions, we were able to further assess the degree to which the CDR3 β contributed to selective microbial recognition. As we found that the TCR β chain was important for the selective recognition of *S. Typhimurium*, but less so for *C. albicans* or BCG, these data would support the hypothesis that the CDR3 β could contribute to the selective recognition of microbial infection. We also observed that, in addition to a broad range of responses to microbial infections, MAIT responses to *E. coli* and *M. smegmatis* are correlated, as are responses to Mtb and *M. marinum*. Furthermore, recent studies have demonstrated that the repertoire of activating ligands for MAIT cells varies between *E. coli* and *M. smegmatis*²⁰.

Prior studies have explored the structural basis by which the MAIT TCR recognizes MR1-bound ligand. The MAIT TCR appears to dock in a conserved mechanism to engage with MR1-bound ligand and the Tyr95 α mediates contact with the ribityl tail of canonical ligands³⁷. However, with recent evidence for non-riboflavin derived ligands binding MR1 to stimulate MAIT cells, the role of other residues in both the TCR α and TCR β chains remains to be determined¹⁸⁻²⁰. Though the variability in reported CDR3 β chains did not contribute to large structural changes in docking of the MAIT TCR, it is possible that CDR3 β hypervariability plays a role in fine-tuning MAIT engagement with antigen, as CDR3 β chains enriched in glycine were reported to make fewer contacts with MR1-bound ligand²⁵. The CDR3 β chain has been reported to display conformational flexibility in its ability to engage with MR1 and its ligand, with the intriguing possibility that distinct ligands can modulate this fine-tuning of the CDR3 β and perhaps increase the potency of TCR contacts with stimulatory ligand^{19, 22, 37}. Differences in the MAIT TCR β chain result in varied affinity between the TCR and MR1-bound ligand. This, combined with a more conserved CDR3 α that also engages with ligand, indicates that both chains are vital in MAIT cell detection of antigen^{24, 38}. Our results support this hypothesis, as all MAIT clones were capable of recognizing and responding to microbial antigen. However, the differences in the CDR3 β region did result in changes in magnitudes of responses, to both microbial infections, and for one pair of clones, to exogenous ligand.

MAIT cells are capable of stimulation through TLR signaling and other non-cognate modes of activation^{6, 34, 39, 40}. However, our results cannot be explained by these non-cognate interactions. A panel of clones that included those described in Figure 1 were originally

shown not to be dependent on TLR signaling, particularly in short term assays, which we have confirmed in follow-up studies with more diverse panels of clones ^{2, 41, 42}. As shown above, we find that the MAIT clone responses to *S. Typhimurum* were similar to those to plate-bound MR1/5-OP-RU, where the only stimulus for MAIT cells is MR1 bound ligand. Thus, while TLR and/or cytokine mediated signaling to activate MAIT cells may well play a role in the context of microbially infected DCs, we show that MR1-mediated ligand recognition is governed, at least in part, by differences in the MAIT TCR.

We would also note that while the responses to 5-OP-RU and *S. Typhimurium* were concordant, we did not observe similar differences to the other microbial species tested. These data, then, would also argue against non-specific T cell activation.

It is possible that stimulation of a T cell clone with differing ligands or microbial infection could result in differential cytokine production. However, in our experience using short term stimulation, we have not observed production of IL-17 or Th2 cytokines in either TCR-dependent or TCR-independent activation of MAIT cells ⁴¹. However, this does not exclude the possibility that cytokines such as IL-17 are being released upon stimulation of these clones. We believe that the use of two APC-free approaches with the MR1/5-OP-RU tetramer, the first direct staining, and the second using the plate bound tetramer to induce T cell activation, support the hypothesis that the MAIT CDR3 β can discriminate between ligand(s). However, these T cell clones could respond to tetramers in a non-cognate fashion. The ability of MAIT cells to respond to cytokines such as IL-12 and IL-18 is well documented and it could be argued that alternate, T cell derived signals could modulate the response to plate-bound tetramer ^{34, 40, 43}. Alternately, it is possible that the equivalent response seen to other microbes reflected dominant, non-cognate interactions. While our experience thus far supports TCR dependence in these short-term assays this remains a possibility ^{2, 26}. Clearly, definition of alternate antigens, or experimental evidence further examining the structural basis of TCR-ligand interactions would be needed to definitively establish the role of the MAIT TCR and CDR3 β in distinguishing between microbial ligand(s).

There has been increasing evidence for diversity among the MR1 ligandome, as well as the ability for individual TCRs to discriminate between these ligands. While MR1-restricted ligands were first identified as metabolites from the riboflavin biosynthesis pathway, recent evidence has shown that ligands can be derived from other pathways or sources and have unique structural features ^{18-20, 38}. Therefore, the MAIT cell specific TCR may recognize a wider array of microbially derived antigens than previously thought and would suggest the possibility that MAIT cells are selective in the recognition of these ligands. Such selectivity would raise the possibility that MAIT cells have immunologic memory.

Recent studies suggest that the MAIT cell-specific TCR repertoire is shaped in response to the wide range of MAIT antigen produced in microbial infection ^{20, 26, 29}. Taken together, these data support the hypothesis that the MAIT cell-specific TCR repertoire adapts in response to exposure to the microbial metabolome.

METHODS

Human subjects.

All blood-derived samples were collected and all experiments were conducted under protocols approved by the institutional review board at Oregon Health and Science University. The bronchoalveolar sample from which the 0033 clones were cultivated was from a patient with active tuberculosis from Durban, South Africa. Bronchoalveolar lavage fluid was obtained under a protocol approved by the University of KwaZulu Natal Human Biomedical Research Ethics Committee and the Partners Institutional Review Board. The participant provided written informed consent.

Cell lines

A549 cells (ATCC CCL-185) were used as stimulators for IFN- γ ELISPOT analysis for MAIT clones, and cultured according to recommended guidelines. Cell lines were confirmed to be mycoplasma free.

Expansion of T-cell clones

T-cell clones were cultured in the presence of X-rayed (3,000 cGray using X-RAD320, Precision X-Ray Inc.) allogeneic PBMCs, X-rayed allogeneic LCL (6,000 cGray) and anti-CD3 monoclonal antibody (20 ng ml⁻¹; Orthoclone OKT3, eBioscience) in RPMI 1640 media with 10% human serum in a T-25 upright flask in a total volume of 30 ml. The cultures were supplemented with IL-2 on days 1, 4, 7 and 10 of culture. The cell cultures were washed on day 5 to remove soluble anti-CD3 monoclonal antibodies.

Sorting and expansion of BAL-derived T cell clones

Cells from bronchoalveolar lavage of Mtb-positive individuals were stained with a viability stain, antibodies against CD4, CD8, TCR $\gamma\delta$, and the MR1/5-OPRu tetramer at optimized concentrations. Cells were sorted on the basis of live CD4⁻ TCR $\gamma\delta$ ⁻, CD8⁺, MR1/5-OPRU tetramer⁺ and limiting dilution was performed as previously described with minor modifications⁴⁴. Briefly, 3 T cells per well were incubated in a 96 well U-bottom plate with X-rayed allogeneic PBMC and LCL, supplemented with IL-2 (5 ng ml⁻¹), IL-7, IL-12, and IL-15 (0.5 ng ml⁻¹), and anti-CD3 monoclonal antibody (30 ng ml⁻¹) to generate MAIT clones, which were tested in an IFN- γ ELISpot to confirm responsiveness to Mtb-infected A549 cells, as well as MR1 restriction. Mtb-responsive MR1-restricted clones were subsequently expanded as described above and used for downstream experiments.

Monocyte-derived DCs

PBMCs obtained by apheresis were resuspended in 2% human serum in RPMI and were allowed to adhere to a T-75 flask at 37 °C for 1 h. After gentle washing twice with PBS, nonadherent cells were removed and 10% human serum in RPMI containing 30 ng ml⁻¹ of IL-4 (Immunex) and 30 ng ml⁻¹ of granulocyte-macrophage colony-stimulating factor (Immunex) was added to the adherent cells. The cells were X-rayed with 3,000 cGray using X-RAD320 (Precision X-Ray Inc.) to prevent cell division. After 5 days, cells were

harvested with cell-dissociation medium (Sigma-Aldrich, Gillingham, UK) and used as APCs in assays.

Microorganisms and preparation of APCs

M. smegmatis, *C. albicans*, *S. Typhimurium*, *E. coli*, *Mtb*, *M. marinum*, and *M. bovis* BCG were utilized from frozen glycerol stock. A549 cells were infected overnight with *M. smegmatis* at a multiplicity of infection (MOI) of 3 at 37 °C. DCs were infected either 2h (*C. albicans*, *S. Typhimurium*, *M. bovis* BCG) or overnight (*M. smegmatis*, *Mtb*, *E. coli*, *M. marinum*) at 37 °C. The MOI and antibiotics used were optimized for APC viability and maximal MR1-restricted response. All infections were performed in the absence of antibiotics, and following the indicated infection time, cells were washed twice in media containing antibiotics, counted, and added to the ELISPOT assay.

Staining and sorting of CD8+ TRAV1-2+ MAIT clones

MAIT clones to be sorted were first incubated in a blocking solution of PBS supplemented with 5% heat inactivated goat serum, 5% heat inactivated pooled human serum, and 0.5% heat inactivated fetal-bovine serum. 1×10^6 MAIT cells were stained with propidium iodide viability stain, and antibodies to CD3, CD4, CD8, TRAV1-2 in the dark at 4 °C for 30 minutes. Samples were then washed with PBS. Live, CD3+, CD4-, CD8+, TRAV1-2+ cells were sorted on an InFlux 11 parameter cell sorter (Becton Dickinson, NJ, USA) with the Oregon Health and Science University flow cytometry core facility. Cells were sorted into RNeasy Lysis Buffer (Qiagen) for further RNA isolation and analysis. Data were analyzed using FlowJo software (TreeStar).

For staining of clones with either CD8 or TRAV1-2, 1×10^6 MAIT clones were incubated in a blocking solution of PBS supplemented with 5% heat-inactivated goat serum, 5% heat-inactivated pooled human serum, and 0.5% heat-inactivated fetal bovine serum. Cells were washed with PBS twice and incubated in the dark for 30 minutes at 4°C with antibodies to either CD8 or TRAV1-2 at manufacturer recommended concentrations. Cells were then washed twice with PBS and acquired on a BD FACS Calibur. Data were analyzed using FlowJo software (TreeStar).

Staining of MAIT clones with tetramer

1×10^6 MAIT clones were first incubated in a blocking solution of PBS supplemented with 5% heat-inactivated goat serum, 5% heat-inactivated pooled human serum, and 0.5% heat-inactivated fetal bovine serum. Cells were incubated with either the MR1/5-OPRU or MR1/6FP tetramer (NIH Tetramer Core, Atlanta, GA, USA) at 0.3 nM at 25µl volume for 45 minutes in the dark. Following this, viability stain (Live Dead Fix Aqua, ThermoFisher, Waltham, MA) and antibodies to CD3, CD4, and CD8 were added at manufacturer recommend concentrations and cells were incubated for 15 min at 4°C in the dark. Cells were washed with PBS and fixed in 1% paraformaldehyde and acquired on a BD Symphony Flow Cytometer.

Molecular analysis of TCR usage.

Clonotypic analysis of sorted cell populations was performed as described previously³¹. In brief, unbiased amplification of all expressed *TRB* or *TRA* gene products was conducted using a template switch-anchored RT-PCR with chain-specific constant region primers. Amplicons were subcloned, sampled, sequenced, and analyzed as described previously⁴⁵. The IMGT nomenclature is used in this study⁴⁶.

CDR3 α sequence similarity

Similarity between CDR3 β sequences was calculated as described previously²⁶. This method allows similarities to be assigned between sequences of different length in an alignment-free manner. An implementation of the similarity matching between CDR3 α sequences is publicly available at http://www.cbs.dtu.dk/services/MAIT_Match. The server takes as input a list of CDR3 α sequences and returns for each a score based on the maximal sequence similarity with a reference database of MAIT cell CDR3 α sequences. A perfect match has a similarity score of 1, and a perfect mismatch a similarity score of 0.

IFN- γ ELISPOT

A MSHA S4510 96 well nitrocellulose-backed plate (Millipore, bought via Fisher Scientific) was coated overnight at 4 °C with 10 $\mu\text{g ml}^{-1}$ solution of anti-IFN- γ monoclonal antibody (Mabtech clone 1-D1K) in a buffer solution of 0.1 M Na₂CO₃, 0.1 M NaHCO₃, pH=9.6). Then, the plate was washed three times with sterile PBS and blocked for 1 h at room temperature with RPMI 1640 media containing 10% heat-inactivated HS pool. Then, the APCs and T cells were prepared as described above and co-incubated overnight. Briefly, DCs or the A549 cell line (all other experiments) were used as APCs at 5×10^3 or 1×10^4 per well in ELISPOT assays. For all blocking ELISPOT assays, APCs were limited to 5×10^3 per well. Where stated, blocking antibodies or antagonists were added for 2 h at 5 $\mu\text{g ml}^{-1}$ (α -HLA-I clone W6/32, and α -MR1 clone 26.5 (Ted Hansen) or appropriate isotype controls). T-cell clones were added at 5×10^3 per well. The plate was incubated overnight at 37 °C and then washed six times with PBS containing 0.05% Tween. The plate was then developed as previously described and analyzed using an AID ELISPOT reader²⁰.

Plate bound tetramer ELISPOT (tetraSPOT) assay

Plate-bound tetramer ELISPOT (tetraSPOT) assay was performed as previously described²⁰. ELISPOT plates were coated with anti-IFN- γ antibody as described above. At the time of coating, MR1 tetramers loaded with either the stimulatory ligand 5-OP-RU or the non-stimulatory ligand 6-FP were also added to wells at amounts between 0 to 0.24 $\mu\text{g ml}^{-1}$ per well. After overnight incubation at 4°C, ELISPOT plates were washed three times with PBS, then blocked with RPMI+10% human serum for 1 hour. 1×10^4 MAIT cell clones were added to wells overnight. IFN- γ ELISPOTs were enumerated following development as previously described²⁰.

Data analysis

Data were analyzed and plotted using Prism 7 GraphPad Software (La Jolla, California). Statistical significance was determined using unpaired Student's two-tailed *t*-test, unless

otherwise indicated. For comparisons of MAIT responses between microbial infection (Figure 1), linear regression analysis was performed and Pearson's correlation coefficient was calculated. For measurements of functional avidity, the curves were transformed to a semilog scale and normalized. Best-fit EC₅₀ was calculated by Prism and statistical significance was determined from differences in Log EC₅₀. Error bars in the figures indicate the standard deviation, standard error of the mean, or the data set range as indicated in each figure legend. *P*-values ≤ 0.05 were considered significant (**P* 0.05; ** *P* 0.01; *** *P* 0.001).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGEMENTS

We thank Pamela Canaday and the OHSU Flow Cytometry Shared Resource for expert assistance. The following reagents were obtained through the NIH Tetramer Core Facility: MR1/5-OP-RU, MR1/6FP tetramers. The MR1/5-OP-RU tetramer technology was developed jointly by J. McCluskey, J. Rossjohn, and D. Fairlie, and the material was produced by the NIH Tetramer Core Facility as permitted to be distributed by the University of Melbourne.

FUNDING

This work was supported by Merit Review Award I01-BX000533 (DML) from the United States Department of Veterans Affairs Biomedical Laboratory Research and resources and the use of facilities at the VA Portland Health Care System; National Institute of Allergy and Infectious Diseases R01-AI048090 (DML); National Heart, Lung, and Blood Institute T32-HL83808 (GAN, EWM); Wellcome Trust Senior Investigator Award 100326/Z/12/Z (DAP); and National Institute of Allergy and Infectious Diseases K08-AI118538 (EBW). Research reported in this publication was supported by the Strategic Health Innovation Partnerships (SHIP) Unit of the South African Medical Research Council (SA MRC) with funds received from the South African Department of Science and Technology as part of a bilateral research collaboration agreement with the Government of India and from an SA MRC Collaborating Centre (ACT4TB/HIV).

REFERENCES

1. Le Bourhis L, Martin E, Peguillet I, Guihot A, Froux N, Core M, et al. Antimicrobial activity of mucosal-associated invariant T cells. *Nat Immunol* 2010; 11:701–8. [PubMed: 20581831]
2. Gold MC, Cerri S, Smyk-Pearson S, Cansler ME, Vogt TM, Delepine J, et al. Human mucosal associated invariant T cells detect bacterially infected cells. *PLoS Biol* 2010; 8:e1000407. [PubMed: 20613858]
3. Treiner E, Duban L, Bahram S, Radosavljevic M, Wanner V, Tilloy F, et al. Selection of evolutionarily conserved mucosal-associated invariant T cells by MR1. *Nature* 2003; 422:164–9. [PubMed: 12634786]
4. Porcelli S, Yockey CE, Brenner MB, Balk SP. Analysis of T cell antigen receptor (TCR) expression by human peripheral blood CD4-8- alpha/beta T cells demonstrates preferential use of several V beta genes and an invariant TCR alpha chain. *J Exp Med* 1993; 178:1–16. [PubMed: 8391057]
5. Gold MC, Eid T, Smyk-Pearson S, Eberling Y, Swarbrick GM, Langley SM, et al. Human thymic MR1-restricted MAIT cells are innate pathogen-reactive effectors that adapt following thymic egress. *Mucosal Immunol* 2013; 6:35–44. [PubMed: 22692454]
6. Wilgenburg BV, Loh L, Chen Z, Pediongco TJ, Wang H, Shi M, et al. MAIT cells contribute to protection against lethal influenza infection in vivo. *Nat Commun* 2018; 9:4706. [PubMed: 30413689]
7. Wang H, D'Souza C, Lim XY, Kostenko L, Pediongco TJ, Eckle SGB, et al. MAIT cells protect against pulmonary *Legionella longbeachae* infection. *Nat Commun* 2018; 9:3350. [PubMed: 30135490]

8. Sakala IG, Kjer-Nielsen L, Eickhoff CS, Wang X, Blazevic A, Liu L, et al. Functional Heterogeneity and Antimycobacterial Effects of Mouse Mucosal-Associated Invariant T Cells Specific for Riboflavin Metabolites. *J Immunol* 2015; 195:587–601. [PubMed: 26063000]
9. Georgel P, Radosavljevic M, Macquin C, Bahram S. The non-conventional MHC class I MR1 molecule controls infection by *Klebsiella pneumoniae* in mice. *Mol Immunol* 2011; 48:769–75. [PubMed: 21190736]
10. Meierovics A, Yankelevich W-JC, Cowley SC. MAIT cells are critical for optimal mucosal immune responses during in vivo pulmonary bacterial infection. *Proc Natl Acad Sci USA* 2013; 110:E3119–28. [PubMed: 23898209]
11. Meierovics AI, Cowley SC. MAIT cells promote inflammatory monocyte differentiation into dendritic cells during pulmonary intracellular infection. *J Exp Med* 2016; 12:2793–2809.
12. Tilloy F, Treiner E, Park S-HH, Garcia C, Lemonnier F, de la Salle H, et al. An Invariant T Cell Receptor Chain Defines a Novel TAP-independent Major Histocompatibility Complex Class Ib-restricted / T Cell Subpopulation in Mammals. *J Exp Med* 1999; 189:1907–21. [PubMed: 10377186]
13. Patel O, Kjer-Nielsen L, Le Nours J, Eckle SBG, Birkinshaw R, Beddoe T, et al. Recognition of vitamin B metabolites by mucosal-associated invariant T cells. *Nat Commun* 2013; 4:2142. [PubMed: 23846752]
14. Corbett AJ, Eckle SBG, Birkinshaw RW, Liu L, Patel O, Mahony J, et al. T-cell activation by transitory neo-antigens derived from distinct microbial pathways. *Nature* 2014; 509:361–5. [PubMed: 24695216]
15. Kjer-Nielsen L, Patel O, Corbett AJ, Le Nours J, Meehan B, Liu L, et al. MR1 presents microbial vitamin B metabolites to MAIT cells. *Nature* 2012; 491:717–23. [PubMed: 23051753]
16. Lepore M, Kalinichenko A, Colone A, Paleja B, Singhal A, Tschumi A, et al. Parallel T-cell cloning and deep sequencing of human MAIT cells reveal stable oligoclonal TCRbeta repertoire. *Nat Commun* 2014; 5:3866. [PubMed: 24832684]
17. Lepore M, Kalinichenko A, Calogero S, Kumar P, Paleja B, Schmalzer M, et al. Functionally diverse human T cells recognize non-microbial antigens presented by MR1. *Elife* 2017; 6.
18. Meermeier EW, Laugel BF, Sewell AK, Corbett AJ, Rossjohn J, McCluskey J, et al. Human TRAV1–2-negative MR1-restricted T cells detect *S. pyogenes* and alternatives to MAIT riboflavin-based antigens. *Nat Commun* 2016; 7:12506. [PubMed: 27527800]
19. Keller AN, Eckle SB, Xu W, Liu L, Hughes VA, Mak JY, et al. Drugs and drug-like molecules can modulate the function of mucosal-associated invariant T cells. *Nat Immunol* 2017.
20. Harrieff MJ, McMurtrey C, Froyd CA, Jin H, Cansler M, Null M, et al. MR1 displays the microbial metabolome driving selective MR1-restricted T cell receptor usage. *Sci Immunol* 2018; 3.
21. Awad W, Ler GJM, Xu W, Keller AN, Mak JYW, Lim XY, et al. The molecular basis underpinning the potency and specificity of MAIT cell antigens. *Nat Immunol* 2020; 21:400–11. [PubMed: 32123373]
22. López-Sagaseta J, Dulberger CL, Crooks JE, Parks CD, Luoma AM, McFedries A, et al. The molecular basis for Mucosal-Associated Invariant T cell recognition of MR1 proteins. *Proc Natl Acad Sci USA* 2013; 110:E1771–8. [PubMed: 23613577]
23. Reantragoon R, Kjer-Nielsen L, Patel O, Chen Z, Illing PT, Bhati M, et al. Structural insight into MR1-mediated recognition of the mucosal associated invariant T cell receptor. *J Exp Med* 2012; 209:761–74. [PubMed: 22412157]
24. Lopez-Sagaseta J, Dulberger CL, McFedries A, Cushman M, Saghatelian A, Adams EJ. MAIT recognition of a stimulatory bacterial antigen bound to MR1. *J Immunol* 2013; 191:5268–77. [PubMed: 24108697]
25. Reantragoon R, Corbett AJ, Sakala IG, Gherardin NA, Furness JB, Chen Z, et al. Antigen-loaded MR1 tetramers define T cell receptor heterogeneity in mucosal-associated invariant T cells. *J Exp Med* 2013; 210:2305–20. [PubMed: 24101382]
26. Gold MC, McLaren JE, Reistetter JA, Smyk-Pearson S, Ladell K, Swarbrick GM, et al. MR1-restricted MAIT cells display ligand discrimination and pathogen selectivity through distinct T cell receptor usage. *J Exp Med* 2014; 211:1601–10. [PubMed: 25049333]

27. Dias J, Leeansyah E, Sandberg JK. Multiple layers of heterogeneity and subset diversity in human MAIT cell responses to distinct microorganisms and to innate cytokines. *Proc Natl Acad Sci USA* 2017; 114:E5434–E43. [PubMed: 28630305]
28. Lepore M, Kalinichenko A, Kalinichenko A, Colone A, Paleja B, Singhal A, et al. Parallel T-cell cloning and deep sequencing of human MAIT cells reveal stable oligoclonal TCR β repertoire. *Nat Commun* 2014; 5:3866. [PubMed: 24832684]
29. Howson LJ, Napolitani G, Shepherd D, Ghadbane H, Kurupati P, Preciado-Llanes L, et al. MAIT cell clonal expansion and TCR repertoire shaping in human volunteers challenged with *Salmonella* Paratyphi A. *Nat Commun* 2018; 9:253. [PubMed: 29343684]
30. Wong EB, Gold MC, Meermeier EW, Xulu BZ, Khuzwayo S, Sullivan ZA, et al. TRAV1-2+ CD8+ T-cells including oligoclonal expansions of MAIT cells are enriched in the airways in human tuberculosis. *Communications Biology* 2019; 2(1).
31. Quigley MF, Almeida JR, Price DA, Douek DC. Unbiased molecular analysis of T cell receptor expression using template-switch anchored RT-PCR. *Curr Protoc Immunol* 2011; 94(1).
32. Shen W-J WH-S, Xiao Q-W, Guo X, Smale S. Towards a mathematical foundation of immunology and amino acid chains. *arXiv preprint arXiv* 2012 Available at <https://arxiv.org/abs/1205.6031>
33. Kurioka A, Ussher JE, Cosgrove C, Clough C, Fergusson JR, Smith K, et al. MAIT cells are licensed through granzyme exchange to kill bacterially sensitized targets. *Mucosal Immunol* 2015; 8:429–40. [PubMed: 25269706]
34. Ussher JE, van Wilgenburg B, Hannaway RF, Ruustal K, Phalora P, Kurioka A, et al. TLR signalling in human antigen-presenting cells regulates MR1-dependent activation of MAIT cells. *Eur J Immunol* 2016; 46(7): 1600–14. [PubMed: 27105778]
35. Bánki Z, Krabbendam L, Klaver D, Leng T, Kruis S, Mehta H, et al. Antibody opsonization enhances MAIT cell responsiveness to bacteria via a TNF-dependent mechanism. *Immunol Cell Biol* 2019; 97:538–51. [PubMed: 30695101]
36. Ioannidou K, Baumgaertner P, Gannon PO, Speiser MF, Allard M, Hebeisen M, et al. Heterogeneity assessment of functional T cell avidity. *Sci Rep* 2017; 7:44320. [PubMed: 28287160]
37. Eckle SBG, Birkinshaw RW, Kostenko L, Corbett AJ, McWilliam HEG, Reantragoon R, et al. A molecular basis underpinning the T cell receptor heterogeneity of mucosal-associated invariant T cells. *J Exp Med* 2014; 211:1585–600. [PubMed: 25049336]
38. Gherardin NA, Keller AN, Woolley RE, Le Nours J, Ritchie DS, Neeson PJ, et al. Diversity of T Cells Restricted by the MHC Class I-Related Molecule MR1 Facilitates Differential Antigen Recognition. *Immunity* 2016; 44:32–45. [PubMed: 26795251]
39. Suliman S, Murphy M, Musvosvi M, Gela A, Meermeier EW, Geldenhuys H, et al. MR1-Independent Activation of Human Mucosal-Associated Invariant T Cells by *Mycobacteria*. *J Immunol* 2019; 203(11): 2917–27. [PubMed: 31611259]
40. Ussher JE, Bilton M, Attwod E, Shadwell J, Richardson R, de Lara C, et al. CD161++ CD8+ T cells, including the MAIT cell subset, are specifically activated by IL-12+IL-18 in a TCR-independent manner. *Eur J Immunol* 2014; 44:195–203. [PubMed: 24019201]
41. Sharma PK, Wong EB, Napier RJ, Bishai WR, Ndung'u T, Kaspirowicz VO, et al. High expression of CD26 accurately identifies human bacteria-reactive MR1-restricted MAIT cells. *Immunology* 2015; 145:443–53. [PubMed: 25752900]
42. Laugel B, Lloyd A, Meermeier EW, Crowther MD, Connor TR, Dolton G, et al. Engineering of Isogenic Cells Deficient for MR1 with a CRISPR/Cas9 Lentiviral System: Tools To Study Microbial Antigen Processing and Presentation to Human MR1-Restricted T Cells. *J Immunol* 2016; 197(3): 971–82 [PubMed: 27307560]
43. Leng T, Akther HD, Hackstein CP, Powell K, King T, Friedrich M, et al. TCR and Inflammatory Signals Tune Human MAIT Cells to Exert Specific Tissue Repair and Effector Functions. *Cell Rep* 2019; 28:3077–91.e5. [PubMed: 31533032]
44. Lewinsohn DM, Briden AL, Reed SG, Grabstein KH, Alderson MR. *Mycobacterium tuberculosis*-reactive CD8+ T lymphocytes: the relative contribution of classical versus nonclassical HLA restriction. *J Immunol* 2000; 165:925–30. [PubMed: 10878367]

45. Price DA, Brenchley JM, Ruff LE, Betts MR, Hill BJ, Roederer M, et al. Avidity for antigen shapes clonal dominance in CD8+ T cell populations specific for persistent DNA viruses. *J Exp Med* 2005; 202:1349–61. [PubMed: 16287711]
46. Lefranc MP, Pommie C, Ruiz M, Giudicelli V, Foulquier E, Truong L, et al. IMGT unique numbering for immunoglobulin and T cell receptor variable domains and Ig superfamily V-like domains. *Dev Comp Immunol* 2003; 27:55–77. [PubMed: 12477501]

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Figure 1: Diversity of MAIT responses to microbially infected DCs

MAIT clones generated by limiting dilution analysis from human PBMC were stimulated overnight with dendritic cells infected with *Mtb*, *M. marinum*, *E. coli*, or *M. smegmatis*. IFN- γ ELISPOT analysis was performed to measure cytokine production by MAIT clones (20000 MAIT cells per well, 20000 APC per well). **(a)** IFN- γ production by MAIT clones in response to all microbial infections, with each spot representing one clone ($n = 42$ for *M. marinum*, $n = 86$ for *E. coli*, $n = 92$ for *M. smegmatis* and *Mtb*). Experiments were performed in duplicate with control clones consistent across experiments **(b)** Scatter plot of comparison of IFN- γ production by MAIT clones from (Fig 1A) between microbial infections. Linear regression analysis was performed and Pearson's correlation coefficient (R) with sample size (n) was calculated and reported for each comparison.

Figure 2: Screening of MAIT clones for TRAV1-2 expression and reactivity to *M. smegmatis*

Example plots (571F3-2) of flow cytometric and IFN- γ ELISPOT analysis of MAIT clones sent for sequencing of the MAIT TCR (a): MAIT clones were stained with antibodies to CD3, CD4, CD8, TRAV1-2, and viability stain. CD8+ TRAV1-2+ clones were sorted for sequencing of the MAIT TCR. Plots are gated on live CD3+, CD4- cells. (b):

Responsiveness of MAIT clones (5×10^3 /well) to A549 cells (5×10^3 /well), incubated 1h with blocking antibodies ($5 \mu\text{g ml}^{-1}$) to MR1 (α -MR1, Clone 26.5), pan-Class I (α -HLA-I, Clone W6-32), or an anti-mouse IgG2a isotype, and infected overnight with *M. smegmatis* (MOI = 3). IFN- γ production was measured by ELISPOT and reported as IFN- γ spot forming units/5000 T cells (IFN- γ SFU). Error bars represent mean and standard error from duplicate wells from a representative experiment. Experiments were repeated >3 times with similar results

Figure 3: IFN- γ production of paired MAIT clones in response to microbial infection
 MAIT cell clone pairs sharing the same CDR3 α and different CDR3 β sequences were stimulated with DCs infected with *C. albicans* (MOI = 3), *S. enterica* serovar Typhimurium (MOI = 30), or *M. bovis* BCG (MOI = 15). IFN- γ ELISPOT was performed as described in Figure 2b. Data are aggregated from 3 independent experiments, which were each performed in duplicate. Error bars represent mean and standard error. Student's *t*-tests were performed to compare IFN- γ responses between paired MAIT clones and P-values < 0.05 were considered significant (**P* 0.05; ***P* 0.01).

Figure 4: Responses of paired MAIT clones to plate bound 5-OP-RU MR1 tetramer

(a) Paired MAIT clones were stimulated with plate-bound MR1/5-OP-RU or the control MR1/6FP tetramer (NIH Tetramer Core, Atlanta, GA, USA) at a range of concentrations as indicated. Modified ELISPOT analysis was performed as described previously to assess IFN- γ production by MAIT clones in response to plate bound tetramer²⁰. Error bars represent means and standard error of technical replicates. Circles represent MR1/5-OP-RU tetramer and squares represent MR1/6FP tetramer. Results are representative of 3 independent experiments. EC₅₀ was calculated and reported for each clone for responsiveness to MR1/5-OP-RU tetramer.

(b) Paired MAIT clones were stained with antibodies for viability, CD3, CD4, CD8, and the MR1/5-OP-RU tetramer or the control MR1/6FP tetramer and assessed by flow cytometry for frequency of MR1/5-OP-RU tetramer positive cells. Gating strategy is shown in Supplemental Figure 1. MR1/6FP control tetramer is shown as a gray histogram. MFI is reported in Table 2.

(c) Paired MAIT clones were stained with an antibody to CD8 and assessed for expression of CD8 by flow cytometry. Unstained cells are shown as gray histograms. MFI is reported in Table 2.

(d) Paired MAIT clones were stained with antibodies for viability and CD3, and live singlet cells were assessed for expression of CD8 by flow cytometry. Gating strategy is shown in Supplemental Figure 1. MFI is reported in Table 2.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Table 1:Paired MAIT clones with identical TCR α and differing TCR β chains

Name	TRAV	CDR3 α	TRAJ	MAIT Match Score	TRBV	CDR3 β	TRBJ
D0033A2	1-2	CAVTDSNYQLI	33	0.9583	3-1	CASSQAETELNTGELF	2-2
D0033A10					3-1	CASSSGLEVTGELF	2-2
D0033A6	1-2	CAVVDSNYQLI	33	0.9587	4-2	CASSHSSGTGGNEQF	2-1
403A9					20-1	CSARDGGEAYNEQF	2-1
450A9	1-2	CAVRDSNYQLI	33	0.9567	20-1	CSAREVEGTYEQY	2-7
571F3-2					6-4	CASSEASGGTDTQY	2-3
450B9	1-2	CAVMDSNYQLI	33	0.9573	6-1	CASTPSGEFSEAF	1-1
427D8-2					5-1	CASSLLRQGTEKLF	1-4

Table 2:

Geometric MFI of T cell clones for Figure 4

Name	MFI 5-OP-RU Tetramer	MFI CD3	MFI CD8
D0033A2	2480	14908	487
D0033A10	2128	15118	687
D0033A6	2619	10872	531
403A9	1604	12767	600
450A9	2028	7091	538
571F3-2	2765	8256	568
450B9	2524	8424	408
427D8-2	3442	10916	655