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REVIEW ARTICLE

MAIT cells as attractive vaccine targets

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Mucosal-associated invariant T (MAIT) cells are a subset of T cells that perform innate-like immunity functions upon recognition of small molecule vitamin B metabolites presented by the MHC, class I-related protein-1 (MR1). MAIT cells are profuse in humans, but especially abundant in blood, liver, lungs, and mucosal layers. The mucosa is a common site of carcinogenesis and MAIT cells have been found in both primary and metastatic tumors. MAIT cells target a host of microbes including *Mycobacterium tuberculosis*, *Staphylococcus aureus*, *Salmonella enterica*, *Legionella longbeachae*, *Escherichia coli*, and *Candida albicans*, and are highly activated in viral infections. Cytokines produced by MAIT cells are both anticancerous and antibacterial, but also have proinflammatory and possibly tumorigenic properties. In addition, it is believed that MAIT cells play a protective role in viral infections in an MR1-independent fashion. Based on our summary of recent advances concerning both MR1-mediated and MR1-independent MAIT cell immune responses, we weigh the strengths and weaknesses of these cells for vaccine development.

Keywords: antitumorigenic; antiviral; bacterial infection; immunomodulation; MAIT cells; therapeutics; vaccines

MAIT cells

The discovery of two highly distinct but cooperative lymphocyte subsets, derived from the thymus (T cells) and the bone marrow (B cells), was the starting point for research to explain the human immune response. T cells are mostly involved in cellular immunity, but crucially help B cells in the production of antibodies. The T cells of the immune system contain membrane-bound T cell receptors (TCRs) which recognize microbial antigens presented by surface proteins on antigen-presenting cells (APCs). The TCRs are heterodimers constructed either from alpha (α) and beta (β) or gamma (γ) and delta (δ) components and are described as an αβ- or a γδ-TCR. The T cells are additionally characterized by the presence or absence of CD8 and CD4 transmembrane co-stimulatory receptors ($CD8^+$ or $^-$ and $CD4^+$ or $^-$) which together with the TCR activate T cells. The conventional αβ-TCR T cells are either $CD8^+$ or $CD4^+$ while unconventional γδ-TCR T cells are almost exclusively defined as lacking the expression of CD8 and CD4 co-receptors. The most common antigens recognized by conventional T cells are peptide metabolites while the unconventional T cells respond to another group of very specific ligands (for recent reviews see refs. [1,2]) (Fig. 1).

A very important and unique subset of unconventional T cells exists in humans, primates, rodents, and ruminants [3–6] that was first discovered in 1993 [7].

Abbreviations
APCs, antigen-presenting cells; ART, antiretroviral therapy; DN, double negative; HCV, hepatitis C virus; MAIT, mucosal-associated invariant T; MMR, measles, mumps, and rubella; *Mtb, Mycobacterium tuberculosis*; NHPs, nonhuman primates; NKG2D, natural-killer group 2, member D; PLZF, promyelocytic leukemia zinc finger; RORγt, retinoic acid-related orphan receptor γt; TB, tuberculosis; TCRs, T cell receptors.

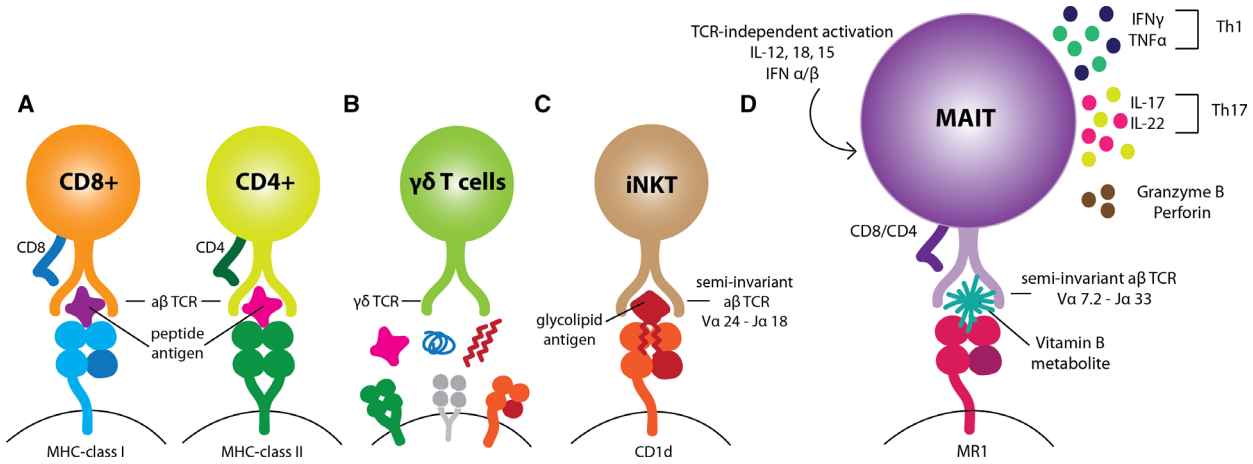

Fig. 1. Schematic representation of the main T cell subsets. (A) Conventional CD8+ and CD4+ T cells recognize the antigen presented through the MHC molecule on the APC by the TCR. (B) γδ T cells are 'unconventional' T cells expressing a γδ TCR which recognizes different ligands presented by diverse receptors (e.g., MHC, CD1, and other surface-bound proteins). (C) The invariant natural killer T (iNKT) cells express an invariant αβ TCR which binds to glycolipid antigens exposed on the MHC class I-like molecule, CD1d. (D) MAIT cells are restricted by MR1 and recognize riboflavin (vitamin B_2) biosynthesis derivatives by the invariant αβ TCR. MAIT cells can also be activated in a TCR-independent manner by various cytokines.

These mucosal-associated invariant T (MAIT) cells make up approximately 10% of all T cells in healthy humans [8]. MAIT cells were first found in the gut mucosa, but are now known to exist in nonmucosal sites, such as the liver, peripheral blood, and lungs as well where they can be enriched to up to 40% of the total T cell number [5,9,10]. Although most MAIT cells display effector memory-like phenotypes [9] that allow for their activation either through both TCR-dependent and -independent processes [11,12], MAIT cells are defined by their semi-invariant TCR containing the invariant Vα7.2 gene segment rearranged typically with a Jα12, 20, or 33 segment, coupled to variable β-chains. These TCR variants as well as the expression of a C-type lectin-like receptor, $CD161^{++}$, and IL-18R, best define human MAIT cells [13]. The semi-invariant TCR of MAIT cells is not activated through peptide- or lipid-based metabolites, as in canonical T cells, but, instead, through the unconventional covalent binding of riboflavin (Vitamin B_2) precursor pyrimidine derivatives by the nonpolymorphic MHC, class I-related protein-1 (MR1) [14–17] (and reviewed in [18]).

MR1-mediated activation

The key pyrimidine derivative, a small nucleoside 5-amino-6-D-ribitylaminouracil (5-A-RU), is a precursor in the riboflavin biosynthetic pathway that is produced by many bacteria and fungi from GTP in four enzymatic steps [14,19–21]. 5-A-RU either enters the microbial riboflavin biosynthesis pathway or reacts nonenzymatically with endogenous dioxo compounds produced in mammalian glycolysis or microbial metabolic pathways to form a profoundly unstable and reactive α-iminocarbonyl adduct 5-(2-oxopropylideneamino)-6-D-ribitylaminouracil (5-OP-RU, shown in Fig. 2A) or 5-(2-oxoethylideneamino-6-D-ribitylamine (5-OE-RU) that can be trapped by a lysine residue on MR1 to form a second Schiff base, thus covalently attaching the molecule to the protein (Fig. 2A) [14]. MR1 is stored in the endoplasmic reticulum in a conformation ready for binding the riboflavin metabolites. Binding triggers a molecular switch that results in the 1,2-diimino−MR1 complex to be trafficked to the APC membrane where it subsequently presents to the MAIT cell TCR [22]. This process has been confirmed by structural elucidation of the MAIT cell TCR−5-OP-RU−MR1 ternary complex in bacteria [14] (reviewed in ref. [23]). Following MR1-mediated activation, MAIT cells rapidly produce a host of cytokines, consistent with a T helper (Th1)-type, Th17-type, or mixed profile, including IFN-γ, TNFs, IL-17, and IL-22. These cytokines, either directly (IFN-γ, TNFs, IL-17), or indirectly through cell proliferation (IL-22), promote cytolysis of the infecting agents (Fig. 1) [9,24–27], hence leading to the control of various bacterial infections in human infectious diseases and in animal models [28]. This work led to the manufacture of commercially available MR1-Ag-tetramers that allow for the conclusive identification of MAIT cells even when these are

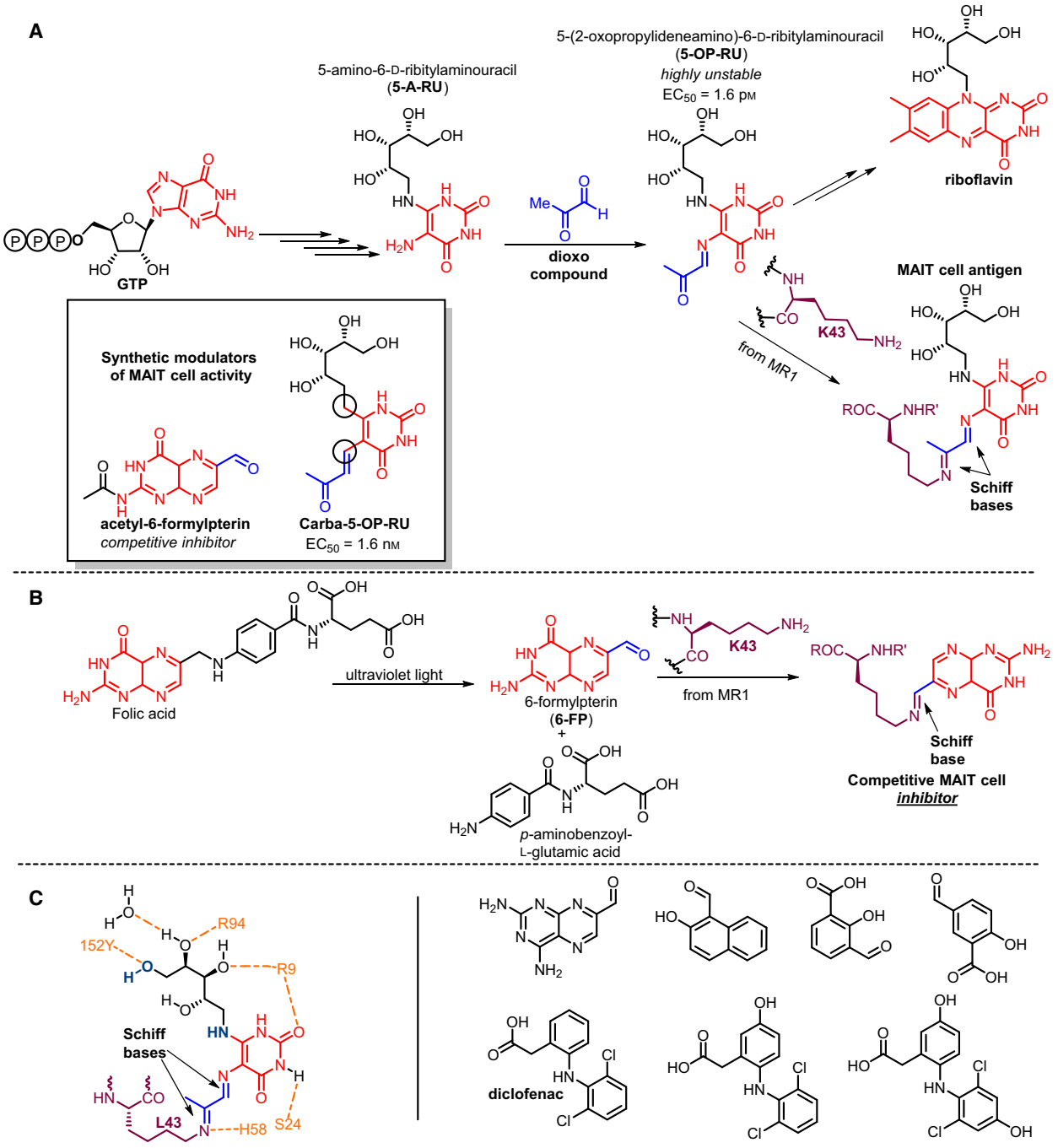

Fig. 2. (A) Partial biosynthesis of 5-OP-RU from GTP. 5-OP-RU is highly reactive and enters the riboflavin biosynthesis pathway or is trapped by MR1 and then presented to MAIT cells, thus inducing an immune response [14]. (B) Photodegradation of folic acid under ultraviolet light to form *p*-aminobenzoyl-L-glutamic acid and 6-FP, the first known modulator of MAIT cell activity [18]. (C) (left) MR1 almost entirely envelops the 5-OP-RU substrate (adapted from ref. 15) [14,15]. (right) Nonsubstrate-based, drug-like modulators of MAIT cell activity indicating promiscuity of the MR1 binding pocket [27].

present in only very low abundance in mammals and humans [14,23,29].

Prior to the discovery of 5-OP-RU, the first ligand ever identified for MR1 was 6-formylpterin (6-FP), an ultraviolet light photodegradation product of folic acid. Interestingly, like 5-A-RU, 6-FP even forms the Schiff base with the formyl moiety of the ε-amino moiety of Lys43 of MR1. However, 6-FP does not activate

MAIT cells – it is, in fact, a competitive inhibitor (Fig. 2B) [30]. Also discovered to be a competitive inhibitor was acetyl-6-formylpterin, a synthetic acylated analogue of 6-FP (Fig. 2A, boxed) [31] (for a review of these discoveries see [18]). MR1 almost entirely envelops the 5-OP-RU molecule as evidenced by the many contacts with the 5-OP-RU molecule and the MR1 binding site (Fig. 2C, left) [14]. Further crystal structures of the MR1 binding pocket have been solved with a series of diverse chemical ligands (even one containing the nonsteroidal anti-inflammatory drug, diclofenac) with limited structural similarity to 5-OP-RU that are either agonists or inhibitors of MAIT cells (Fig. 2C, right) [32]. It has been posited whether or not these nonsubstrate molecules activate or inhibit MAIT cells depends on TCRβ chain usage [32,33]. The MR1 binding pocket clearly presents a high degree of promiscuity and this suggests that none of the critical structural components of 5-OP-RU, even the double Schiff base, have been confirmed. It is hypothesized that more, yet to be discovered, antigens for MAIT cells could exist [29,33–35].

As a result of the MR1 binding pocket promiscuity and the high instability of 5-OP-RU and 5-OE-RU, significant efforts have been made to identify more stable analogues with the same stimulatory ability, but as yet have been met with only limited success. The most stable analogue (carba-5-OP-RU, Fig. 2A, boxed) is still a 1000 times less potent antigen (EC_{50} = 1.6 nM) than 5-OP-RU (EC_{50} = 1.6 pM) and is only stable in an aqueous environment [15]. Nonetheless, this seminal work marks appreciable progress in realizing artificial mediation of MAIT cell activity.

Human MAIT cells divide primarily into two functionally different subsets characterized by the presence ($CD8^+CD4^-$, major phenotype) or absence ($CD8^-CD4^-$, double negative (DN), minor phenotype) of the CD8α co-receptor for the TCR. Although 95% of human MAIT cells do not bear the CD4 phenotype [36], $CD4^+$ MAIT cells exist and are upregulated in active tuberculosis infection [12,37]. Initially, due to the high level of conservation of the MR1 protein and the small number of riboflavin-based activators it was thought that the MAIT cell response would be homogeneous regardless of the microbial infectant. Now it is known that MAIT cells actually provide microbe-specific responses with layered heterogeneity [38]. This difference will have very important implications in infectious and inflammatory diseases as MAIT cells bearing different phenotypes are able to effect differential responses in a pathogen-specific manner, although further research is required.

TCR-independent activation

Some functions of MAIT cells can be activated by innate inflammatory and antiviral cytokines, most commonly IL-12 and IL-18 [11], but also IL-15, IFN-α/β [39], in an MR1-independent mechanism [11] and can concurrently augment MAIT cell's TCR-dependent activation [40,41]. TCR-independent activation is the primary mode of action for MAIT cells in combating viral infection mainly through secretion of cytokine IFN-γ [42] and serine protease granzyme B [39]. The implications of MR1-independent MAIT cell activation are tremendous as this response provides utility for MAIT cells beyond antimicrobial effects and into viral infection, including influenza [43], HIV [44], and hepatitis B and C [45] to be discussed.

Vaccines

Successful vaccines initiate long-term protection against pathogens by inducing adaptive immunity. In addition to the principle epitope, they also typically contain one or more adjuvants, an entity added to the formulation to boost the immune response. Vaccines have had a profound impact on human health and have resulted in the eradication of the smallpox virus [46]. In general terms, two types of vaccines exist. The first category are the vaccines to treat existing debilitations, termed therapeutic vaccines. The second category contains vaccines that are delivered in order to prevent the development of a certain ailment, termed preventative vaccines. Synthetic or semi-synthetic bioconjugate epitopes offer a promising alternative to isolated conjugates in vaccines because absolute structural integrity can be confirmed as well as a greater degree of purity in the final product achieved. These two factors offer the advantage of mitigating or potentially suppressing hyporesponsiveness or side-effects of the vaccine in patients [47,48].

General considerations

Currently available vaccines target B cells to produce neutralizing antibodies; they do not generate protective T cell responses. B cells recognize only native antigens that have not been processed stipulating that B cells have extreme specificity. This is very good if the antigens of the pathogen remain unchanged, as in the case of the measles, mumps, and rubella (MMR) vaccine where the B cells are immunogenic against the conserved, essential proteins of the virus. However, in

influenza (more detail below), for example, the available vaccines only result in immunogenicity from B cells against the hemagglutinin (H) and the neuraminidase (N) viral surface proteins which mutate rapidly and broadly, thus having limited utility [49]. This makes the adaptive T cells attractive in vaccine design, in general, because they can recognize processed antigens.

Most vaccines are administered to patients intramuscularly or intradermally and may not confer and maintain good mucosal immunity. This is especially important in the cases of intestinal, respiratory or urogenital tract infections. The possibility for MAIT cells to be exploited in mucosal vaccine design is an attractive direction for exploration as they may be harnessed as adjuvants to boost systemic vaccine efficacy [33,50]. Mature MAIT cells express a $CD95^{hi}CD62L^{lo}CD45RO^{+}CD45RA^{lo}CD27^{+}CD122^{+}$ memory-like phenotype in the blood [51–53] and may display transcription factors retinoic acid-related orphan receptor γt (RORγt), promyelocytic leukemia zinc finger (PLZF), T-bet, and Helios [36,53,54]. The MAIT cell frequency is low in young children [9,53,55]. It has been posited that by adding MAIT cell antigens in combination with toll-like receptor antigens to vaccines their immunogenicity in infants may improve [56].

The success of vaccine development hinges upon the selection of an appropriate animal model for testing and analysis during the preclinical phase. The natural abundancy of MAIT cells in mice is one-tenth that of humans [51,57] and could be potentially prohibitively low. Highly encouragingly, the MR1 protein in nonhuman primates (NHPs) and humans function comparably, such that NHPs are a useful preclinical modal for MAIT cell-targeted vaccines [6]. Here, we discuss the potential for MAIT cells to be explored as vaccine targets in bacterial and viral infection as well as tumorigenesis.

MAIT cells in vaccines against microbial infection

In broad terms, MAIT cells have been implicated in a host of microbial infections and as a result could be attractive vaccine targets especially as they serve as the first mode of defense against certain bacterial infections [28]. MAIT cells are cytotoxic [58–60] and can respond to some superantigens in the absence of ligand binding [3]. It has been proposed that MAIT cell-mediated cytotoxicity can be induced, augmented, or reestablished to play a protective role in microbial infections [24,61–63]. We analyze this potential in more detail below.

Staphylococcus aureus

Staphylococcus aureus is a major human pathogen responsible for a host of life-threatening clinical infections including bacteremia and infective endocarditis [64] and is still linked to up to 50,000 deaths in the United States each year [65]. In *S. aureus* infection, dendritic cells could increase the expression of the activation marker CD69 on MAIT cells by >100-fold [66], however, these MAIT cells can produce IFN-γ which has powerful antimicrobial properties [8,24]. Interestingly, it has been shown that *S. aureus* superantigens are able to circumvent MR1-dependent activation pathway and stimulate MAIT cells in the absence of ligand binding. Subsequent challenge results in MAIT cell exhaustion and anergy rendering them unable to respond to MR1-restricted antigens. It is also possible that MAIT cell propagation is reduced or abated during *S. aureus* infection [3]. Although the exhaustion resulting from these superantigens may prevent MAIT cells from further propagation of their response, if this shortcoming can be circumvented in the future MAIT cells would be very useful in fighting *S. aureus* infections and be valuable in vaccine design [8].

Escherichia coli and *Candida albicans*

Escherichia coli, although the most studied microorganism worldwide, still poses very serious public health problems [67]. *Candida albicans*, a ubiquitous mammalian fungus, is the most prevalent fungal pathogen in humans [68]. In general, MAIT cells have been shown to respond to both infections in an MR1-dependent manner as would be expected [24]. Nonetheless, pioneering work has shown that despite the high level of conservation of the MR1 protein, MAIT cells display transcriptionally and phenotypically distinct subsets with differing, but specific activity profiles [36,38]. MAIT cell effector responses toward *E. coli* and *C. albicans* demonstrate disparate MR1 dependency and TCR β-chain bias indicating that MAIT cells may have differential sub-specificities for the antigens originating from these two organisms (functional heterogeneity). MAIT cell responses were still consistent with an effector memory-like profile ($CD45RO^{+}CD127^{+}CD62L^{lo}$) [33,38]. These results are encouraging for vaccine design: if 'tailor-made' responses to either infectious agent can be induced in the future, MAIT cells could serve as powerful adjuvants in both preventative and therapeutic vaccines against these pathogens.

Mycobacterium tuberculosis

Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (*Mtb*), was one of the top 10 causes of death in 2016 and more than 10 million new cases are thought to present each year [69]. The lack of an effective vaccine is a contributing factor to mortality [70]. Conflicting data have been published, but two recent studies suggest that in both active, early [37] and latent [71] *Mtb* infection, MAIT cell frequency of some phenotypes is enhanced or unchanged. In active pulmonary *Mtb* infection, $CD4^+$ MAIT cells, the very minor MAIT cell phenotype, along with γδ T cells were enriched and the $CD8^+$ or DN phenotypes unchanged. Individuals that were exposed, but not infected with *Mtb* presented high MAIT cell CD25 expression and granzyme B production coupled with a depressed CD69 and IFN-γ response. Evidently, it is this phenotype that offers resistance to the bacterium. A final observation suggests that MAIT cell abundance and reactivity correlates with gut microbiome composition. Apparently, MAIT cell responses in an *Mtb* infection could be controlled by the microbiome of the intestine [37].

In latent infections, MAIT cell frequency in peripheral blood was increased, however, the phenotype differed. In latent infections the $CD8^+CD4^-$ MAIT cell phenotype dominated. Clearly further research is needed to determine the importance of phenotype in treating latent and active infection, however, MAIT cells could provide successful immune control of *Mtb* infection [71] and are attractive for vaccine design. When rhesus macaques are subjected to Bacillus Calmette-Guerin vaccination at 14 days postinfection at the vaccine site, MAIT cells were more frequent in the skin at the vaccination site compared to the skin at a distal site. MAIT cells in rhesus macaques were activated and respond quickly to direct *Mtb* infection [6]. One potential drawback we note is that MAIT cell accumulation in the lungs, the actual site of infection, during *Mtb* infection is limited [72], but may not be important for vaccine design. These studies make a very strong case for incorporating MAIT cells into *Mtb* vaccines.

Salmonella enterica and *Legionella longbeachae*

Salmonella enterica is a Gram-negative bacterium and many of its serotypes are a prominent source of community-acquired infections among low- and middle-income families. Multi-drug resistance to *Salmonella* serotypes is becoming a serious concern in antibiotic treatment [73]. *Legionella longbeachae* is also a Gram-negative bacterium and is the principal causative agent of Legionnaire's disease in some countries, including New Zealand, Australia, and Thailand. Legionnaire's disease is a serious, potentially deadly, atypical form of pneumonia with increasing prevalence [74]. In both *S. enterica* [54] and *L. longbeachae* [56] infections in mice, MAIT cells accumulate at the site of infection in the lungs in an MR1-dependent fashion. MAIT cell frequency in the mice was augmented by exposure to the 5-OP-RU ligand before the mice were infected with *L. longbeachae* one month later, essentially screening MAIT cells as potential adjuvants. The bacterial load was significantly lower in wild-type mice that had been previously exposed to 5-OP-RU. This is the first encouraging example of actually examining 5-OP-RU to serve as a vaccine [56].

Potential pitfalls

Helicobacter pylori is a common infecting agent affecting approximately half the world's population with a much higher prevalence in developing countries and individuals living in poor socio-economic conditions. It is also the main cause of chronic gastritis and peptic ulcers [75]. The scenario for MAIT cells in *H. pylori* infections appears to be more complicated and debatable than in many of the other microbial infections, as it appears they are not solely protective. Using a murine *H. pylori* SS1 infection model it has recently been shown using MR1 tetramers that MAIT cells may actually contribute to chronic gastritis. Following the infection of mice, MAIT cells accumulated in high numbers in the gastric mucosa and presented a memory Th1/Th17 effector phenotype resulting in augmented secretion of the pro-inflammatory cytokine, IL-17. The increased levels of this cytokine resulted in accelerated gastritis via increased recruitment of immunogenic cells (i.e., neutrophils, macrophages, dendritic cells, eosinophils, and non-MAIT T cells) and gastric atrophy [76,77]. Because *H. pylori* is prevalent in the human gut microbiome, augmenting MAIT cell levels as a result of a vaccine may result in gastric illnesses in some individuals that carry this bacterium. The role of MAIT cells in this disease certainly requires further studies.

Overall, there are clear advantages to investigating MAIT cells in vaccine development. We believe that harnessing the anti-microbial ability of MAIT cells could contribute to the design of potent immunotherapies and vaccines against highly resilient infectious agents that remain global public health threats [78]. Coupled with their effector memory-like characteristics and ability to accumulate and remain at the site of (at least some) infections they are attractive candidates. Development must take into account the possibly pro-

inflammatory implications in the gut, as a result of IL-17A efflux that can be a limiting factor if MAIT cell recruitment really is mediated by the gut microbiome [37]. Gastric perturbations may arise as a highly undesirable side effect.

MAIT cells in vaccines against viral infections

Another burgeoning area of research is MAIT cell implication in viral infections. A series of recent studies underscore the potential utility of MAIT cells in antiviral vaccines as many viral infections exhaust MAIT cell levels. Viral infections stimulate MAIT cells in an MR1-independent manner, instead responding to cytokine profiles released dependent on the infecting agent.

Hepatitis B virus

Hepatitis B viral infection is a major global threat with an estimated 240 million people chronically infected and another 780 000 individuals dying each year due to complications of the virus, including hepatic cirrhosis, hepatocellular carcinoma, and chronic liver failure [79]. Despite the fact that a preventative vaccine for HBV does exist, a small percentage of people can be chronically infected. Furthermore, current prophylactic vaccines are largely ineffective in patients already chronically infected with HBV [80]. In chronic HBV infections, the percentage of cytokine-producing T cells and MAIT cells were significantly depleted in number and the level of intracellular expression of granzyme B and IFN-γ were significantly reduced compared to healthy controls [81] due to increased PD-1 levels [45], a regulatory cell-surface immune suppressing protein [82]. If MAIT cell numbers can be artificially increased as an adjuvant in a vaccine during chronic HBV infection, vaccine efficacy could increase dramatically. Further research to this end is needed.

Hepatitis C virus

Hepatitis C virus (HCV) is a member of the *Flaviviridae* family of positive-sense RNA viruses [39]. HCV global prevalence is estimated at 1.2 and 1.7% among adults with an estimated 500 000 deaths attributed to the infection in 2015 [83]. MAIT cell populations in blood are the most severely reduced during chronic HCV infection. The MAIT cells that are present display a phenotype indicative of immune suppression, as a result of high levels of PD-1 expression, and high levels of activation indicated by a high manifestation of granzyme B, HLA-DR, and CD69. They also exhibited altered transcription factor expression and dampened responsiveness to MR1-mediated antigen stimulation. MAIT cell depletion appears to be irreversible even after HCV clearance [84] and blood MAIT cell frequency measurements during HCV mono-infection and HCV/HIV co-infection confirmed this severe irreversible depletion [85]. However, these studies did not address hepatic MAIT cell levels at the site of infection. Hepatic levels of MAIT cells are much higher than that of the blood in infected patients, however, they were still significantly depleted when compared to hepatic MAIT cell levels of uninfected patients. Although their supply is depleted, their level is restored upon 12-week antiviral therapy and their TCR-dependent response remains unaltered [86]. Lower levels of MAIT cells during HCV infection were found, but the cells were still activated by IL-18 from the host response as a result of the virus in a TCR-independent fashion with upregulated expression of granzyme B. MAIT cells were believed to contribute to the immunopathology and host defense [39].

HIV

Despite monumental advances in antiretroviral therapy (ART) in the past decades, infection and death from HIV, especially in developing nations, remains a major health concern [87]. MAIT cell implication in HIV infection is a very hot research topic as discussed below.

In HIV infection, much like in chronic HBV and HCV, MAIT cell levels in the blood are depleted and display a different cytokine receptor profile over uninfected individuals [13,88–90]. This loss of MAIT cell frequency has been demonstrated in simian immunodeficiency virus infection in rhesus macaques as well [91]. Moreover, it is postulated that this functional impairment and decline in MAIT cell populations is caused by systemic chronic immune activation that often accompanies HIV-1 infection [92]. Initially this depletion was thought to be irreversible, however, it has been shown recently that IL-7 administration to chronically HIV-1 infected patients as part of ART can restore MAIT cell peripheral blood levels and function and was more pronounced in the $CD8^+$ subset [93]. Evidence suggests that reconstitution of MAIT cell populations in HIV-1 patients would have a positive effect on the HIV-1 disease progression and transmission [44]. These findings have important implications for vaccine design. HIV-1 infection is associated with an increased incidence of active *Mtb*, even among patients with viral suppression [71,94]. Activation or recruitment of MAIT cells may prevent *Mtb* infections. Hence, an *Mtb* vaccine in combination with

IL-7 therapy could prove to be an efficacious HIV treatment. Rhesus macaques may be an excellent model to test such a therapy.

Mucosal-associated invariant T cell-based antimicrobial vaccines can still be effective, or, at least not detrimental, during chronic viral infections. This finding is crucial as microbial infection is a key source of morbidity and mortality in individuals with chronic viral infections [94]. Therefore, in principle, an antibacterial vaccine targeting MAIT cells can still be highly beneficial during these viral infections. Additionally, MAIT cells may be effective in an actual HIV vaccine if they contribute to host defense as postulated.

Acute viral infections

Influenza, an acute airborne respiratory illness, is still a significant health threat, especially to those aged over 65, pregnant women, and immunocompromised patients. Influenza A and B, of the *orthomyxoviridae* family of viruses cause severe seasonal epidemics [95]. Currently available preventative influenza vaccines provide only strain-specific efficacy. They are currently only able to offer limited cross-protection against seasonal, pandemic, or avian-derived viruses [49].

As in the other viruses, MAIT cell frequency in the peripheral blood of infected patients is reduced which could result in compromised antibacterial immunity increasing the threat of bacterial co-infection, especially *Mtb* [49,96]. However, much like in the chronic infections, in acute viral infections like influenza (and also dengue virus), MAIT cells are specifically and readily activated by cytokines IL-12, -15, -18 and IFN Type 1 in a TCR-independent mechanism [39]. This activation is quantified by measuring IFN-γ secretion by the MAIT cells. In influenza *Mtb* co-infection, MAIT cell activation is assessable. The authors state that these findings are consistent with a possible therapeutic strategy involving MAIT cells [96]. Further research found that despite the low frequency of MAIT cells in mice, very similar findings were discovered in murine models infected with two strains of the influenza A virus. Again, MAIT cells offer protection in respiratory viral infections and are a viable target in therapeutic strategies [97,98]. A murine model may be suitable for testing vaccine strategies involving MAIT cells facilitating their development despite their low natural abundance.

In both chronic and acute viral infections the positive effects for incorporating MAIT cells in vaccine design appear to overshadow the negatives. Although many mechanistic questions remain, increasing MAIT cell activation appears either beneficial or neutral in the worst case. Evidenced by the large volume of MAIT cell-related studies published in recent years it certainly seems that numerous research groups also believe that the therapeutic potential MAIT cells can be harnessed, or should certainly be investigated further. Since the most current studies suggest that MAIT cell numbers in peripheral blood appear invariably suppressed in viral infections, a therapeutic vaccine design with the ability to reconstitute peripheral blood levels of MAIT cells using an optimized cocktail of cytokines appears to be a good goal. However, it is crucial to determine whether the underlying causes for this depression are. It would also be interesting to determine if the compromised MR1-mediated MAIT cell stimulation during the viral infections could be reconstituted or the underlying causes could be irrefutably determined to facilitate their incorporation into a therapeutic vaccine. It is encouraging that in viral infections MAIT cell suppression in rhesus macaques is consistent with that of humans. These primates, therefore, potentially provide a viable model and could facilitate testing MAIT cells in therapeutic designs.

MAIT cells in vaccines against cancer

Cancer vaccines, like other vaccines, can be distinguished into two categories, therapeutic and preventative. A very limited number of therapeutic cancer vaccines have been approved by the US Food and Drug Administration, while no purely cancer-preventative vaccines are currently on the market. The therapeutic effects of the commercially available preventative vaccines have been considered unsatisfactory [99]. Further studies into both vaccine types open new opportunities.

Therapeutic cancer vaccines are administered to patients with the goal of boosting the patient's own immune response by employing suitable antigens to modulate $CD8^+$ T cell-mediated responses. Superficially, MAIT cells are a highly desirable target for therapeutic cancer vaccine design as the primary phenotype for MAIT cells is $CD8^+$, the reality, however, is more complicated. Activated MAIT cells can produce a Th1-type, Th17-type, or a mixed cytokine profile largely dependent on their mode of stimulation, tissue location, and the cytokine environment in which they are found. Induction of a Th17-type profile as in adipose tissue of obese patients, IL-17 will be excreted in elevated levels [100]. Unfortunately, IL-17A elicits pro-tumorigenic responses that can influence the clinical cancer prognosis [12,101]. The pro-tumorigenic and immunosuppressive effects of IL-17A appear to be a limiting factor in therapeutic design [12], but the situation is not as bleak as it may seem. MAIT cells only elicit a Th17-type response from certain tissues under

specific conditions. In the blood and liver, MAIT cells produce insignificant amounts of IL-17A under physiological conditions.

Mucosal-associated invariant T cells of all phenotypes rapidly produce large amounts of IFN-γ, a powerful cytokine that displays cytostatic, proapoptotic, and immunostimulatory properties – all crucial anti-tumorigenic effects. MAIT cells also express high levels of natural-killer group 2, member D (NKG2D), a C-type lectin receptor expressed by other immune cells (e.g., other $CD8^+$ T cells), that is implicated in anti-cancer immune surveillance [102]. MAIT cell's ability to use the activating NKG2D receptor suggests that MAIT cells can engage malignant cells and may potentially carry the ability to eliminate tumor cells, either directly or indirectly [12].

MAIT cell levels in the liver, an organ where non-Th17-type profile displaying MAIT cells reside, of patients with colorectal liver metastasis were only marginally decreased [103]. Should MAIT cell numbers be augmented in some way, they likely would have anti-cancerous properties in this scenario.

Despite many findings implicating MAIT cells in carcinomas, much remains to be discovered (as reviewed in [12]). The innate properties of MAIT cells may be rendered useful in therapeutic vaccines for several reasons: First, they are found in the mucosa, the primary place of malignant development for mucosal-associated cancers. Second, MAIT cells are detectable within metastatic cancers [103], however, their expression of IFN-γ is suppressed [104]. Finally, peripheral blood and the liver contain a high frequency of MAIT cells, common places for would-be metastatic tumors to circulate in an afflicted patient [9,12]. With these characteristics in mind, the amplification of MAIT cells themselves, or their release of anti-tumorigenic cytokines such as IFN-γ, could very likely be useful in therapeutic cancer vaccinations. Moreover, although preventative cancer vaccines are still in their infancy, the effector memory-like characteristics of MAIT cells would render them potent adjuvants in such a therapy.

Feasibility of incorporating MAIT cells into vaccines

The implications of MAIT cells in many infections and diseases and how they can be specifically harnessed in each debilitation to serve as an effective vaccine target have been discussed. However, it is important to present conceptually how MAIT cells can be exploited in an actual vaccine formulation.

Superficially, it appears easier to target MAIT cells in an antimicrobial vaccine, either therapeutic or preventative. We believe that their ability to serve primarily as adjuvant is the greatest, however, their effector memory-like phenotypes provide further potential. To serve as adjuvant in microbial infections, the discovery of a more stable ligand than 5-A-RU, 5-OP-RU, or R-OE-RU would be of a profound benefit. Should such an analogue be developed, it could be added to the vaccine serum directly and administered with the principal epitope for the targeted bacterial infection. Thus, both the acquired immune response arising from the epitope and innate (-like) immunity stemming from the MAIT cells could be augmented in one formulation. Unfortunately, to date, the development of such analogues has thus far proven elusive. We await further developments.

In viral infection, we believe that the current data most strongly support the exploitation of MAIT cells in a therapeutic vaccine and could serve as both the antiviral agent and offer concurrent prevention against bacterial co-infection. As discussed, MAIT cell levels are severely depleted and can show a compromised MR1-dependent response in viral infections. The ability to reconstitute their numbers somehow during administration of a therapeutic vaccine would be highly beneficial. In HIV-1, the administration of recombinant IL-7 has been shown to restore MAIT cells levels in peripheral blood as part of ART [93]. It would certainly be worth determining if the addition of IL-7 to a vaccine serum could possibly induce the same effect.

In malignancies, research implicating MAIT cells is still in its preliminary stages, but a very encouraging fact is that MAIT cells found in the blood and liver, common places for metastasis, produce insignificant amounts of the pro-tumorigenic cytokine IL-17A [12]. An artificial method to increase MAIT cell numbers as part of a therapeutic vaccine is worth investigating. MAIT cells could have the potential to reduce or abate metastasis and should protect against bacterial co-infection in the cancerous patient. Although no such methods to achieve this augmentation are currently known, we posit that a more stable antigen than the known vitamin B_2 metabolites added to the serum may provide such an effect.

Concluding remarks and future directions

An overwhelming body of research supports further investigations into the use of MAIT cells for use in vaccines and in therapeutics and has been explicitly stated in at least one study [56]. In bacterial infections, seminal work describing MAIT cell's ability to produce differential responses depending on the infecting agent [36] and their ability to specifically accumulate

at the site of infection in response to a specific pathogen [54] are very encouraging factors. In viral infections, MAIT cells appear beneficial and could prove especially valuable in protecting against bacterial co-infection in immunocompromised patients as evidenced by studies in patients with HIV-1/*Mtb* co-infections. Insights into the role of MAIT cells in cancer are still rudimentary, but combating metastasis by exploiting elevated MAIT cell levels in the liver and the peripheral blood for adjuvant effects.

Naturally, the pro-inflammatory implications of MAIT cells in the gut of *H. pylori*-infected patients and their potential to produce the pro-tumorigenic and pro-inflammatory cytokine IL-17A cannot be ignored and must be analyzed when formulating a vaccine model. Only very recently the implications for MAIT cells in autoimmune disorders began to be explored. Although, they are generally found to be active and enriched in these pathologies ([37], and references therein), most data are inconclusive and conflicting – studies available in a list of autoimmune disorders suggest MAIT cells can be both inflammatory and protective. These studies have recently been reviewed in [105,106]. Further research is certainly required in this arena of pathologies.

The most critical factor in whether or not MAIT cells are beneficial or deleterious in a pathology is their cytokine profile when activated during a particular disease or stage within the disease. Certainly fine-tuning of MAIT cell behavior is required if they are to be useful for vaccine developments, but with a vast body of evidence implicating protective roles in a wide range of diseases, we are optimistic that such immunomodulation is a worthwhile endeavor.

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References

1 Kumar BV, Connors TJ and Farber DL (2018) Human T Cell Development, Localization, and Function throughout Life. *Immunity* **48**, 202–213.
2 Pasman L and Kasper DL (2017) Building conventions for unconventional lymphocytes. *Immunol Rev* **279**, 52–62.
3 Sandberg JK, Norrby-Teglund A and Leeansyah E (2017) Bacterial deception of MAIT cells in a cloud of superantigen and cytokines. *PLoS Biol* **15**, e2003167.
4 Goldfinch N, Reinink P, Connelley T, Koets A, Morrison I and Van Rhijn I (2010) Conservation of mucosal associated invariant T (MAIT) cells and the MR1 restriction element in ruminants, and abundance of MAIT cells in spleen. *Vet Res* **41**, 62.
5 Huang S, Martin E, Kim S, Yu L, Soudais C, Fremont DH, Lantz O and Hansen TH (2009) MR1 antigen presentation to mucosal-associated invariant T cells was highly conserved in evolution. *Proc Natl Acad Sci USA* **106**, 8290–8295.
6 Greene JM, Dash P, Roy S, McMurtrey C, Awad W, Reed JS, Hammond KB, Abdulhaqq S, Wu HL, Burwitz BJ, *et al.* (2017) MR1-restricted mucosal-associated invariant T (MAIT) cells respond to mycobacterial vaccination and infection in nonhuman primates. *Mucosal Immunol* **10**, 802–813.
7 Porcelli S, Yockey CE, Brenner MB and Balk SP (1993) Analysis of T cell antigen receptor (TCR) expression by human peripheral blood $CD4^{-}8^{-}$ α/β T cells demonstrates preferential use of several Vβ genes and an invariant TCR α chain. *J Exp Med* **178**, 1–16.
8 O'Brien EC and McLoughlin RM (2019) Considering the 'Alternatives' for next-generation anti-*Staphylococcus aureus* vaccine development. *Trends Mol Med* **25**, 171–184.
9 Dusseaux M, Martin E, Serriari N, Péguillet I, Premel V, Louis D, Milder M, Le Bourhis L, Soudais C, Treiner E, *et al.* (2011) Human MAIT cells are xenobiotic-resistant, tissue-targeted, $CD161^{hi}$ IL-17-secreting T cells. *Blood* **117**, 1250–1259.
10 Le Bourhis L, Mburu YK and Lantz O (2013) MAIT cells, surveyors of a new class of antigen: development and functions. *Curr Opin Immunol* **25**, 174–180.
11 Ussher JE, Bilton M, Attwod E, Shadwell J, Richardson R, de Lara C, Mettke E, Kurioka A, Hansen TH, Klenerman P, *et al.* (2014) $CD161^{++}CD8^{+}$ T cells, including the MAIT cell subset, are specifically activated by IL-12+IL-18 in a TCR-independent manner. *Eur J Immunol* **44**, 195–203.
12 Haeryfar SMM, Shaler CR and Rudak PT (2018) Mucosa-associated invariant T cells in malignancies: a faithful friend or formidable foe? *Cancer Immunol Immunother* **67**, 1885–1896.
13 Juno JA, Phetsouphanh C, Klenerman P and Kent SJ (2019) Perturbation of mucosal-associated invariant T cells and iNKT cells in HIV infection. *Curr Opin HIV AIDS* **14**, 77–84.
14 Corbett AJ, Eckle SB, Birkinshaw RW, Liu L, Patel O, Mahony J, Chen Z, Reantragoon R, Meehan B, Cao H, *et al.* (2014) T-cell activation by transitory neo-antigens derived from distinct microbial pathways. *Nature* **509**, 361–365.
15 Mak JY, Xu W, Reid RC, Corbett AJ, Meehan BS, Wang H, Chen Z, Rossjohn J, McCluskey J, Liu L, *et al.* (2017) Stabilizing short-lived Schiff base

derivatives of 5-aminouracils that activate mucosal-associated invariant T cells. *Nat Commun* **8**, 14599.
16 Eckle SBG, Corbett AJ, Keller AN, Chen ZJ, Godfrey DI, Liu LG, Mak JYW, Fairlie DP, Rossjohn J and McCluskey J (2015) Recognition of vitamin B precursors and byproducts by mucosal associated invariant T cells. *J Biol Chem* **290**, 30204–30211.
17 Kjer-Nielsen L, Patel O, Corbett AJ, Le Nours J, Meehan B, Liu LG, Bhati M, Chen ZJ, Kostenko L, Reantragoon R, *et al*. (2012) MR1 presents microbial vitamin B metabolites to MAIT cells. *Nature* **491**, 717–723.
18 Kjer-Nielsen L, Corbett AJ, Chen Z, Liu L, Mak JY, Godfrey DI, Rossjohn J, Fairlie DP, McCluskey J and Eckle SB (2018) An overview on the identification of MAIT cell antigens. *Immunol Cell Biol* **96**, 573–587.
19 Bacher A and Lingens F (1969) The structure of the purine precursor in riboflavin biosynthesis. *Angew Chem Int Ed* **8**, 371–372.
20 Bacher A and Mailander B (1973) Biosynthesis of riboflavin. The structure of the purine precursor. *J Biol Chem* **248**, 6227–6231.
21 Mailander B and Bacher A (1976) Biosynthesis of riboflavin. Structure of the purine precursor and origin of the ribityl side chain. *J Biol Chem* **251**, 3623–3628.
22 McWilliam HE, Eckle SB, Theodossis A, Liu L, Chen Z, Wubben JM, Fairlie DP, Strugnell RA, Mintern JD, McCluskey J, *et al*. (2016) The intracellular pathway for the presentation of vitamin B-related antigens by the antigen-presenting molecule MR1. *Nat Immunol* **17**, 531–537.
23 D'Souza C, Chen Z and Corbett AJ (2018) Revealing the protective and pathogenic potential of MAIT cells. *Mol Immunol* **103**, 46–54.
24 Le Bourhis L, Martin E, Peguillet I, Guihot A, Froux N, Core M, Levy E, Dusseaux M, Meyssonnier V, Premel V, *et al*. (2010) Antimicrobial activity of mucosal-associated invariant T cells. *Nat Immunol* **11**, 701–708.
25 Gibbs A, Leeansyah E, Introini A, Paquin-Proulx D, Hasselrot K, Andersson E, Broliden K, Sandberg JK and Tjernlund A (2017) MAIT cells reside in the female genital mucosa and are biased towards IL-17 and IL-22 production in response to bacterial stimulation. *Mucosal Immunol* **10**, 35–45.
26 Leeansyah E, Loh L, Nixon DF and Sandberg JK (2014) Acquisition of innate-like microbial reactivity in mucosal tissues during human fetal MAIT-cell development. *Nat Commun* **5**, 3143.
27 Dudakov JA, Hanash AM and van den Brink MR (2015) Interleukin-22: immunobiology and pathology. *Annu Rev Immunol* **33**, 747–785.
28 Salou M, Franciszkiewicz K and Lantz O (2017) MAIT cells in infectious diseases. *Curr Opin Immunol* **48**, 7–14.
29 Reantragoon R, Corbett AJ, Sakala IG, Gherardin NA, Furness JB, Chen Z, Eckle SB, Uldrich AP, Birkinshaw RW, Patel O, *et al*. (2013) Antigen-loaded MR1 tetramers define T cell receptor heterogeneity in mucosal-associated invariant T cells. *J Exp Med* **210**, 2305–2320.
30 Patel O, Kjer-Nielsen L, Le Nours J, Eckle SB, Birkinshaw R, Beddoe T, Corbett AJ, Liu L, Miles JJ, Meehan B, *et al*. (2013) Recognition of vitamin B metabolites by mucosal-associated invariant T cells. *Nat Commun* **4**, 2142.
31 Eckle SB, Birkinshaw RW, Kostenko L, Corbett AJ, McWilliam HE, Reantragoon R, Chen Z, Gherardin NA, Beddoe T, Liu L, *et al*. (2014) A molecular basis underpinning the T cell receptor heterogeneity of mucosal-associated invariant T cells. *J Exp Med* **211**, 1585–1600.
32 Keller AN, Eckle SBG, Xu WJ, Liu LG, Hughes VA, Mak JYW, Meehan BS, Pediongco T, Birkinshaw RW, Chen ZJ, *et al*. (2017) Drugs and drug-like molecules can modulate the function of mucosal-associated invariant T cells. *Nat Immunol* **18**, 402–411.
33 Xiao X and Cai J (2017) Mucosal-associated invariant T cells: new insights into antigen recognition and activation. *Front Immunol* **8**, 1540.
34 Franciszkiewicz K, Salou M, Legoux F, Zhou Q, Cui Y, Bessoles S and Lantz O (2016) MHC class I-related molecule, MR1, and mucosal-associated invariant T cells. *Immunol Rev* **272**, 120–138.
35 Meermeier EW, Laugel BF, Sewell AK, Corbett AJ, Rossjohn J, McCluskey J, Harriff MJ, Franks T, Gold MC and Lewinsohn DM (2016) Human TRAV1-2-negative MR1-restricted T cells detect *S. pyogenes* and alternatives to MAIT riboflavin-based antigens. *Nat Commun* **7**, 12506.
36 Dias J, Leeansyah E and Sandberg JK (2017) Multiple layers of heterogeneity and subset diversity in human MAIT cell responses to distinct microorganisms and to innate cytokines. *Proc Natl Acad Sci USA* **114**, E5434–E5443.
37 Vorkas CK, Wipperman MF, Li K, Bean J, Bhattarai SK, Adamow M, Wong P, Aubé J, Juste MAJ, Bucci V, *et al*. (2018) Mucosal-associated invariant and γδ T cell subsets respond to initial *Mycobacterium tuberculosis* infection. *JCI Insight* **3**, e121899.
38 Dias J, Boulouis C, Gorin JB, van den Biggelaar R, Lal KG, Gibbs A, Loh L, Gulam MY, Sia WR, Bari S, *et al*. (2018) The $CD4^{-}CD8^{-}$ MAIT cell subpopulation is a functionally distinct subset developmentally related to the main CD^{+} MAIT cell pool. *Proc Natl Acad Sci USA* **115**, E11513–E11522.
39 van Wilgenburg B, Scherwitzl I, Hutchinson EC, Leng T, Kurioka A, Kulicke C, de Lara C, Cole S, Vasanawathana S, Limpitikul W, *et al*. (2016) MAIT cells are activated during human viral infections. *Nat Commun* **7**, 11653.

40 Slichter CK, McDavid A, Miller HW, Finak G, Seymour BJ, McNevin JP, Diaz G, Czartoski JL, McElrath MJ, Gottardo R, *et al.* (2016) Distinct activation thresholds of human conventional and innate-like memory T cells. *JCI Insight* **1**, e86292.
41 Ussher JE, van Wilgenburg B, Hannaway RF, Ruustal K, Phalora P, Kurioka A, Hansen TH, Willberg CB, Phillips RE and Klenerman P (2016) TLR signaling in human antigen-presenting cells regulates MR1-dependent activation of MAIT cells. *Eur J Immunol* **46**, 1600–1614.
42 Ussher JE, Willberg CB and Klenerman P (2018) MAIT cells and viruses. *Immunol Cell Biol* **96**, 630–641.
43 Loh L, Wang ZF, Sant S, Koutsakos M, Jegaskanda S, Corbett AJ, Liu LG, Fairlie DP, Crowe J, Rossjohn J, *et al.* (2016) Human mucosal-associated invariant T cells contribute to antiviral influenza immunity via IL-18-dependent activation. *Proc Natl Acad Sci USA* **113**, 10133–10138.
44 Cocker ATH, Greathead L, Herasimtschuk AA, Mandalia S, Kelleher P and Imami N (2019) Short communication: Therapeutic immunization benefits mucosal-associated invariant T cell recovery in contrast to interleukin-2, granulocyte-macrophage colony-stimulating factor, and recombinant human growth hormone addition in HIV-1+ treated patients: individual case reports from phase I trial. *AIDS Res Hum Retroviruses* **35**, 306–309.
45 Yong YK, Saeidi A, Tan HY, Rosmawati M, Enström PF, Al Batran R, Vasuki V, Chattopadhyay I, Murugesan A, Vignesh R, *et al.* (2018) Hyper-expression of PD-1 is associated with the levels of exhausted and dysfunctional phenotypes of circulating $CD161^{++}$TCR $iV\alpha7.2^{+}$ mucosal-associated invariant T cells in chronic hepatitis B virus infection. *Front Immunol* **9**, 472.
46 Streefland P, Chowdhury AM and Ramos-Jimenez P (1999) Patterns of vaccination acceptance. *Soc Sci Med* **49**, 1705–1716.
47 Anish C, Schumann B, Pereira CL and Seeberger PH (2014) Chemical biology approaches to designing defined carbohydrate vaccines. *Chem Biol* **21**, 38–50.
48 Parameswarappa SG, Reppe K, Geissner A, Ménová P, Govindan S, Calow ADJ, Wahlbrink A, Weishaupt MW, Monnanda BP, Bell RL, *et al.* (2016) A semi-synthetic oligosaccharide conjugate vaccine candidate confers protection against *Streptococcus pneumoniae* serotype 3 infection. *Cell Chem Biol* **23**, 1407–1416.
49 Nüssing S, Sant S, Koutsakos M, Subbarao K, Nguyen THO and Kedzierska K (2018) Innate and adaptive T cells in influenza disease. *Front Med* **12**, 34–47.
50 Napier RJ, Adams EJ, Gold MC and Lewinsohn DM (2015) The role of mucosal associated invariant T cells in antimicrobial immunity. *Front Immunol* **6**, 344.
51 Rahimpour A, Koay HF, Enders A, Clanchy R, Eckle SB, Meehan B, Chen Z, Whittle B, Liu L, Fairlie DP, *et al.* (2015) Identification of phenotypically and functionally heterogeneous mouse mucosal-associated invariant T cells using MR1 tetramers. *J Exp Med* **212**, 1095–1108.
52 Sakala IG, Kjer-Nielsen L, Eickhoff CS, Wang X, Blazevic A, Liu L, Fairlie DP, Rossjohn J, McCluskey J, Fremont DH, *et al.* (2015) Functional heterogeneity and antimycobacterial effects of mouse mucosal-associated invariant T cells specific for riboflavin metabolites. *J Immunol* **195**, 587–601.
53 Koay HF, Gherardin NA, Enders A, Loh L, Mackay LK, Almeida CF, Russ BE, Nold-Petry CA, Nold MF, Bedoui S, *et al.* (2016) A three-stage intrathymic development pathway for the mucosal-associated invariant T cell lineage. *Nat Immunol* **17**, 1300–1311.
54 Chen Z, Wang H, D'Souza C, Sun S, Kostenko L, Eckle SB, Meehan BS, Jackson DC, Strugnell RA, Cao H, *et al.* (2017) 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.
55 Ben Youssef G, Tourret M, Salou M, Ghazarian L, Houdouin V, Mondot S, Mburu Y, Lambert M, Azarnoush S, Diana JS, *et al.* (2018) Ontogeny of human mucosal-associated invariant T cells and related T cell subsets. *J Exp Med* **215**, 459–479.
56 Wang H, D'Souza C, Lim XY, Kostenko L, Pediongco TJ, Eckle SBG, Meehan BS, Shi M, Wang N, Li S, *et al.* (2018) MAIT cells protect against pulmonary *Legionella longbeachae* infection. *Nat Commun* **9**, 3350.
57 Ghazarian L, Caillat-Zucman S and Houdouin V (2017) Mucosal-associated invariant T cell interactions with commensal and pathogenic bacteria: potential role in antimicrobial immunity in the child. *Front Immunol* **8**, 1837.
58 Le Bourhis L, Dusseaux M, Bohineust A, Bessoles S, Martin E, Premel V, Core M, Sleurs D, Serriari NE, Treiner E, *et al.* (2013) MAIT cells detect and efficiently lyse bacterially-infected epithelial cells. *PLoS Pathog* **9**, e1003681.
59 Kurioka A, Ussher JE, Cosgrove C, Clough C, Fergusson JR, Smith K, Kang YH, Walker LJ, Hansen TH, Willberg CB, *et al.* (2015) MAIT cells are licensed through granzyme exchange to kill bacterially sensitized targets. *Mucosal Immunol* **8**, 429–440.
60 Leeansyah E, Svärd J, Dias J, Buggert M, Nyström J, Quigley MF, Moll M, Sonnerborg A, Nowak P and Sandberg JK (2015) Arming of MAIT cell cytolytic antimicrobial activity is induced by IL-7 and defective in HIV-1 infection. *PLoS Pathog* **11**, e1005072.
61 Rudak PT, Choi J and Haeryfar SMM (2018) MAIT cell-mediated cytotoxicity: Roles in host defense and

therapeutic potentials in infectious diseases and cancer. *J Leukoc Biol* **104**, 473–486.
62 Meierovics A, Yankelevich WJ and Cowley SC (2013) MAIT cells are critical for optimal mucosal immune responses during in vivo pulmonary bacterial infection. *Proc Natl Acad Sci USA* **110**, E3119–3128.
63 Gold MC, Cerri S, Smyk-Pearson S, Cansler ME, Vogt TM, Delepine J, Winata E, Swarbrick GM, Chua WJ, Yu YY, *et al*. (2010) Human mucosal associated invariant T cells detect bacterially infected cells. *PLoS Biol* **8**, e1000407.
64 Tong SY, Davis JS, Eichenberger E, Holland TL and Fowler VG Jr (2015) *Staphylococcus aureus* infections: epidemiology, pathophysiology, clinical manifestations, and management. *Clin Microbiol Rev* **28**, 603–661.
65 Schlecht LM, Peters BM, Krom BP, Freiberg JA, Hänsch GM, Filler SG, Jabra-Rizk MA and Shirtliff ME (2015) Systemic *Staphylococcus aureus* infection mediated by *Candida albicans* hyphal invasion of mucosal tissue. *Microbiology* **161**, 168–181.
66 Cui Y, Franciszkiewicz K, Mburu YK, Mondot S, Le Bourhis L, Premel V, Martin E, Kachaner A, Duban L, Ingersoll MA, *et al*. (2015) Mucosal-associated invariant T cell-rich congenic mouse strain allows functional evaluation. *J Clin Invest* **125**, 4171–4185.
67 Vila J, Sáez-López E, Johnson JR, Römling U, Dobrindt U, Cantón R, Giske CG, Naas T, Carattoli A, Martínez-Medina M, *et al*. (2016) *Escherichia coli*: an old friend with new tidings. *FEMS Microbiol Rev* **40**, 437–463.
68 Noble SM, Gianetti BA and Witchley JN (2017) *Candida albican*s cell-type switching and functional plasticity in the mammalian host. *Nat Rev Microbiol* **15**, 96–108.
69 Meier NR, Jacobsen M, Ottenhoff THM and Ritz N (2018) A Systematic Review on Novel *Mycobacterium tuberculosis* Antigens and Their Discriminatory Potential for the Diagnosis of Latent and Active Tuberculosis. *Front Immunol* **9**, 2476.
70 Mangtani P, Abubakar I, Ariti C, Beynon R, Pimpin L, Fine PE, Rodrigues LC, Smith PG, Lipman M, Whiting PF, *et al*. (2014) Protection by BCG vaccine against tuberculosis: a systematic review of randomized controlled trials. *Clin Infect Dis* **58**, 470–480.
71 Paquin-Proulx D, Costa PR, Terrassani Silveira CG, Marmorato MP, Cerqueira NB, Sutton MS, O'Connor SL, Carvalho KI, Nixon DF and Kallas EG (2018) Latent *Mycobacterium tuberculosis* Infection Is Associated With a Higher Frequency of Mucosal-Associated Invariant T and Invariant Natural Killer T Cells. *Front Immunol* **9**, 1394.
72 Kauffman KD, Sallin MA, Hoft SG, Sakai S, Moore R, Wilder-Kofie T, Moore IN, Sette A, Arlehamn CSL and Barber DL (2018) Limited Pulmonary Mucosal-Associated Invariant T Cell Accumulation and Activation during *Mycobacterium tuberculosis* Infection in Rhesus Macaques. *Infect Immun* **86**, e00431–18.
73 Eng SK, Pusparajah P, Ab Mutalib NS, Ser HL, Chan KG and Lee LH (2015) Salmonella: A review on pathogenesis, epidemiology and antibiotic resistance. *Front Life Sci* **8**, 284–293.
74 Cunha BA, Burillo A and Bouza E (2016) Legionnaires' disease. *Lancet* **387**, 376–385.
75 Gravina AG, Zagari RM, De Musis C, Romano L, Loguercio C and Romano M (2018) *Helicobacter pylori* and extragastric diseases: A review. *World J Gastroenterol* **24**, 3204–3221.
76 D'Souza C, Pediongco T, Wang H, Scheerlinck JY, Kostenko L, Esterbauer R, Stent AW, Eckle SBG, Meehan BS, Strugnell RA, *et al*. (2018) Mucosal-Associated Invariant T Cells Augment Immunopathology and Gastritis in Chronic *Helicobacter pylori* Infection. *J Immunol* **200**, 1901–1916.
77 Blosse A, Lehours P, Wilson KT and Gobert AP (2018) Helicobacter: Inflammation, immunology, and vaccines. *Helicobacter* **23** (Suppl 1), e12517.
78 Moreira ML, Tsuji M, Corbett AJ, Araújo MSS, Teixeira-Carvalho A, Martins-Filho OA, Peruhype-Magalhães V and Coelho-dos-Reis JG (2017) MAIT-cells: A tailor-made mate in the ancient battle against infectious diseases? *Immunol Lett* **187**, 53–60.
79 Lozano R, Naghavi M, Foreman K, Lim S, Shibuya K, Aboyans V, Abraham J, Adair T, Aggarwal R, Ahn SY, *et al*. (2012) Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet* **380**, 2095–2128.
80 Vandepapelière P, Lau GK, Leroux-Roels G, Horsmans Y, Gane E, Tawandee T, Merican MI, Win KM, Trepo C, Cooksley G, *et al*. (2007) Therapeutic vaccination of chronic hepatitis B patients with virus suppression by antiviral therapy: a randomized, controlled study of co-administration of HBsAg/AS02 candidate vaccine and lamivudine. *Vaccine* **25**, 8585–8597.
81 Yong YK, Tan HY, Saeidi A, Rosmawati M, Atiya N, Ansari AW, Rajarajeswaran J, Vadivelu J, Velu V, Larsson M, *et al*. (2017) Decrease of CD69 levels on TCR Vα7.2$^+$CD4$^+$ innate-like lymphocytes is associated with impaired cytotoxic functions in chronic hepatitis B virus-infected patients. *Innate Immun* **23**, 459–467.
82 Syn NL, Teng MWL, Mok TSK and Soo RA (2017) De-novo and acquired resistance to immune checkpoint targeting. *Lancet Oncol* **18**, e731–e741.
83 Kish T, Aziz A and Sorio M (2017) Hepatitis C in a New Era: A Review of Current Therapies. *P T* **42**, 316–329.
84 Hengst J, Strunz B, Deterding K, Ljunggren HG, Leeansyah E, Manns MP, Cornberg M, Sandberg JK, Wedemeyer H and Björkström NK (2016) Nonreversible MAIT cell-dysfunction in chronic

hepatitis C virus infection despite successful interferon-free therapy. *Eur J Immunol* **46**, 2204–2210.
85 Beudeker BJB, van Oord GW, Arends JE, Schulze zur Wiesch J, van der Heide MS, de Knegt RJ, Verbon A, Boonstra A and Claassen MAA (2018) Mucosal-associated invariant T-cell frequency and function in blood and liver of HCV mono- and HCV/HIV co-infected patients with advanced fibrosis. *Liver Int* **38**, 458–468.
86 Bolte FJ, O'Keefe AC, Webb LM, Serti E, Rivera E, Liang TJ, Ghany M and Rehermann B (2017) Intra-Hepatic Depletion of Mucosal-Associated Invariant T Cells in Hepatitis C Virus-Induced Liver Inflammation. *Gastroenterology* **153** (1392–1403), e1392.
87 Maartens G, Celum C and Lewin SR (2014) HIV infection: epidemiology, pathogenesis, treatment, and prevention. *Lancet* **384**, 258–271.
88 Cosgrove C, Ussher JE, Rauch A, Gartner K, Kurioka A, Huhn MH, Adelmann K, Kang YH, Fergusson JR, Simmonds P, *et al*. (2013) Early and nonreversible decrease of $CD161^{++}$ /MAIT cells in HIV infection. *Blood* **121**, 951–961.
89 Spaan M, Hullegie SJ, Beudeker BJ, Kreefft K, van Oord GW, Groothuismink ZM, van Tilborg M, Rijnders B, de Knegt RJ, Claassen MA, *et al*. (2016) Frequencies of Circulating MAIT Cells Are Diminished in Chronic HCV, HIV and HCV/HIV Co-Infection and Do Not Recover during Therapy. *PLoS One* **11**, e0159243.
90 Eberhard JM, Kummer S, Hartjen P, Hüfner A, Diedrich T, Degen O, Lohse AW, van Lunzen J and Schulze zur Wiesch J (2016) Reduced $CD161^{+}$ MAIT cell frequencies in HCV and HIV/HCV co-infection: Is the liver the heart of the matter? *J Hepatol* **65**, 1261–1263.
91 Vinton C, Wu F, Rossjohn J, Matsuda K, McCluskey J, Hirsch V, Price DA and Brenchley JM (2016) Mucosa-associated invariant T cells are systemically depleted in simian immunodeficiency virus-infected Rhesus Macaques. *J Virol* **90**, 4520–4529.
92 Leeansyah E, Ganesh A, Quigley MF, Sönnerborg A, Andersson J, Hunt PW, Somsouk M, Deeks SG, Martin JN, Moll M, *et al*. (2013) Activation, exhaustion, and persistent decline of the antimicrobial MR1-restricted MAIT-cell population in chronic HIV-1 infection. *Blood* **121**, 1124–1135.
93 Sortino O, Richards E, Dias J, Leeansyah E, Sandberg JK and Sereti I (2018) IL-7 treatment supports $CD8^{+}$ mucosa-associated invariant T-cell restoration in HIV-1-infected patients on antiretroviral therapy. *AIDS* **32**, 825–828.
94 Saeidi A, Tien Tien VL, Al-Batran R, Al-Darraji HA, Tan HY, Yong YK, Ponnampalavanar S, Barathan M, Rukumani DV, Ansari AW, *et al*. (2015) Attrition of TCR $V\alpha7.2^{+}CD161^{++}$ MAIT cells in HIV-tuberculosis co-infection is associated with elevated levels of PD-1 expression. *PLoS One* **10**, e0124659.
95 Paules C and Subbarao K (2017) *Influenza*. *Lancet* **390**, 697–708.
96 Mendy J, Jarju S, Heslop R, Bojang AL, Kampmann B and Sutherland JS (2018) Changes in *Mycobacterium tuberculosis*-Specific Immunity With Influenza co-infection at Time of TB Diagnosis. *Front Immunol* **9**, 3093.
97 van Wilgenburg B, Loh L, Chen Z, Pediongco TJ, Wang H, Shi M, Zhao Z, Koutsakos M, Nüssing S, Sant S, *et al*. (2018) MAIT cells contribute to protection against lethal influenza infection in vivo. *Nat Commun* **9**, 4706.
98 Hinks TSC, van Wilgenburg B, Loh L, Chen Z, Pediongco T, Wang H, Shi M, Zhao Z, Koutsakos M, Nüssing S, *et al*. (2018) MAIT cells contribute to a protective antiviral innate response to influenza infection. *Eur Respir J* **52**, PA5459.
99 Ye Z, Qian Q, Jin H and Qian Q (2018) Cancer vaccine: learning lessons from immune checkpoint inhibitors. *J Cancer* **9**, 263–268.
100 Carolan E, Tobin LM, Mangan BA, Corrigan M, Gaoatswe G, Byrne G, Geoghegan J, Cody D, O'Connell J, Winter DC, *et al*. (2015) Altered distribution and increased IL-17 production by mucosal-associated invariant T cells in adult and childhood obesity. *J Immunol* **194**, 5775–5780.
101 Tosolini M, Kirilovsky A, Mlecnik B, Fredriksen T, Mauger S, Bindea G, Berger A, Bruneval P, Fridman WH, Pagès F, *et al*. (2011) Clinical impact of different classes of infiltrating T cytotoxic and helper cells (Th1, Th2, Treg, Th17) in patients with colorectal cancer. *Cancer Res* **71**, 1263–1271.
102 López-Soto A, Huergo-Zapico L, Acebes-Huerta A, Villa-Alvarez M and Gonzalez S (2015) NKG2D signaling in cancer immunosurveillance. *Int J Cancer* **136**, 1741–1750.
103 Shaler CR, Tun-Abraham ME, Skaro AI, Khazaie K, Corbett AJ, Mele T, Hernandez-Alejandro R and Haeryfar SMM (2017) Mucosa-associated invariant T cells infiltrate hepatic metastases in patients with colorectal carcinoma but are rendered dysfunctional within and adjacent to tumor microenvironment. *Cancer Immunol Immunother* **66**, 1563–1575.
104 Sundstrom P, Ahlmanner F, Akéus P, Sundquist M, Alsen S, Yrlid U, Borjesson L, Sjöling A, Gustavsson B, Wong SB, *et al*. (2015) Human Mucosa-Associated Invariant T Cells Accumulate in Colon Adenocarcinomas but Produce Reduced Amounts of IFN-γ. *J Immunol* **195**, 3472–3481.
105 Chiba A, Murayama G and Miyake S (2018) Mucosal-Associated Invariant T Cells in Autoimmune Diseases. *Front Immunol* **9**, 1333.
106 Rouxel O and Lehuen A (2018) Mucosal-associated invariant T cells in autoimmune and immune-mediated diseases. *Immunol Cell Biol* **96**, 618–629.