Contents lists available at ScienceDirect

Biochemical Pharmacology

journal homepage: www.elsevier.com/locate/biochempharm

Commentary

Harnessing the antibacterial and immunological properties of mucosal-associated invariant T cells in the development of novel oral vaccines against enteric infections

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ARTICLE INFO

Article history:

Received 18 July 2014

Accepted 15 August 2014

Available online xxx

Keywords:

Vaccine

Adjuvant

MAIT cell

Mucosal

Enteric

ABSTRACT

Enteric infections are a major cause of mortality and morbidity with significant social and economic implications worldwide and particularly in developing countries. An attractive approach to minimizing the impact of these diseases is *via* the development of oral vaccination strategies. However, oral vaccination is challenging due to the tolerogenic and hyporesponsive nature of antigen presenting cells resident in the gastrointestinal tract. The inclusion of adjuvants in oral vaccine formulations has the potential to overcome this challenge. To date no oral adjuvants have been licenced for human use and thus oral adjuvant discovery remains a key goal in improving the potential for oral vaccine development. Mucosal-associated invariant T (MAIT) cells are a recently discovered population of unconventional T cells characterized by an evolutionarily conserved $\alpha\beta$ T cell receptor (TCR) that recognizes antigens presented by major histocompatibility complex (MHC) class I-related (MR1) molecule. MAIT cells are selected intra-thymically by MR1 expressing double positive thymocytes and enter the circulation with a naïve phenotype. In the circulation they develop a memory phenotype and are programmed to home to mucosal tissues and the liver. Once resident in these tissues, MAIT cells respond to bacterial and yeast infections through the production of chemokines and cytokines that aid in the induction of an adaptive immune response. Their abundance in the gastrointestinal tract and ability to promote adaptive immunity suggests that MAIT cell activators may represent attractive novel adjuvants for use in oral vaccination.

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1. Introduction

Enteric infections represent a substantial economic and social burden worldwide. Globally, although mainly in sub-Saharan Africa and south and south-east Asia, diarrheal disease is responsible for one in every ten fatalities in children under the age of five [1]. In African and Asian children the main causes of

moderate-to-severe diarrhoea are: rotavirus, *Cryptosporidium*, Enterotoxigenic *Escherichia coli* (ETEC) producing heat-stable toxin, *Shigella*, *Aeromonas*, *Vibrio cholerae* O1, and *Campylobacter jejuni* [2]. Beyond the risk of death as a result of acute symptoms there can also be long term consequences of these infections including: Guillain–Barre syndrome, reactive arthritis and haemolytic uraemic syndrome. Chronic enteric infections due to *Helicobacter pylori* affect up to 50% of the world's population, and increase the risk of ulcers and gastric cancers in adults. Therefore, due to the extensive burden of enteric infections, novel strategies to protect against these enteric pathogens are crucial, especially in developing countries where there is a higher risk of infection.

Vaccination involves the administration of antigenic material to stimulate adaptive immunity, thus conferring lasting protection against infectious diseases. To date the majority of vaccines are administered by injection and this has been successful for a number of pathogens, however there are a number of benefits to developing mucosally administered vaccines (reviewed by Freytag and Clements [3]). Firstly, many pathogens gain access *via* the

Abbreviations: APC, antigen-presenting cell; CT, cholera toxin; CDR, complementarity-determining region; CB, cord blood; DC, dendritic cell; DN, double negative; DP, double positive; ETEC, enterotoxigenic *Escherichia coli*; GIT, gastrointestinal tract; LT, heat-labile enterotoxin of *E. coli*; IgA, immunoglobulin A; ICU, intensive care unit; IFN γ , interferon gamma; IL, interleukin; IEC, intraepithelial cell; IEL, intraepithelial lymphocyte; iNKT, invariant natural killer T; LP, lamina propria; LVS, live vaccine strain; MHC, major histocompatibility complex; MR1, MHC class I-related; MAIT, mucosal associated invariant T; TCR, T cell receptor; Th, T helper; TB, tuberculosis; TNF, tumour necrosis factor; vita-PAMPS, viability-associated PAMPS.

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<http://dx.doi.org/10.1016/j.bcp.2014.08.017>

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mucosal surfaces; therefore, directly targeting the entry site is attractive. In general, systemic immunization is ineffective or suboptimal at promoting sustained mucosal immunity. Secondly, a needle free approach is beneficial for a number of reasons including: reduced pain and stress, decreased potential for contracting diseases through contaminated needles and a reduced requirement for medically trained personnel to immunize. Thus making it a more economic and sustainable vaccination method in poorer countries. Thirdly, it has been suggested that optimally formulated mucosal vaccines could have further economic and practical benefits due to improved stability, longer shelf life and decreased requirements for cold storage which would improve the possibility for mass vaccination, especially in developing countries.

Despite these clear benefits and growing interest in developing oral vaccines, progress has been slow. This is partly due to difficulties overcoming the combination of physiological, physical and chemical barriers present in the gastrointestinal tract (GIT) (acidic pH, tight junctions, peristalsis, proteases, bile and mucus). Although these barriers are effective in providing host protection, they also make the development and successful delivery of oral vaccines challenging. Despite these obstacles a handful of oral vaccines are currently licenced for human use, these include: Oral polio vaccine (polio), Vivotif[®] (typhoid fever), Rotarix[®], RotaTeq[®] (rotavirus), Shanchol[™], Dukoral[®] and mORC-vax[™] (cholera). In many cases the effectiveness of whole cell killed and subunit oral vaccines is lower in endemic regions than that seen in trials carried out in developed countries and in travellers. This is due to poorer immunogenicity in local populations. This phenomenon is often referred to as the tropical barrier, and is likely the result of nutritional deficiencies, differences in microflora composition and parasites [4]. Future vaccines against enteric diseases should, therefore, focus on being immunogenic in residents of impoverished countries, and cost effective, to allow for mass immunization in high risk regions.

The tolerogenic and hypo-responsive environment of the GIT is also a difficult challenge to overcome and increases the need for adjuvants in whole cell killed and subunit oral vaccine formulations. The primary role of adjuvants in vaccine formulations is to activate the innate immune system. This in turn triggers the induction of adaptive immunity, which is essential for protection against infectious diseases. Experimental oral adjuvants such as ADP-ribosylating enterotoxins (cholera toxin (CT) and the heat-labile enterotoxin of *E. coli* (LT)), have shown very promising results in animal models [5,6]. It has also been reported that synthetic oligodeoxynucleotides containing unmethylated CpG dinucleotides (CpG ODN), and monophosphoryl lipid A (MPL) have mucosal adjuvant properties [3]. CT has been the gold standard experimental oral adjuvant for many decades and it has been a very useful tool in demonstrating the protective potential of oral vaccination. Unfortunately, its toxicity precludes it from human use in its native form. Attempts to reduce the toxicity of CT and LT while maintaining their adjuvanticity have shown some promise and these derivatives remain among the leading potential oral adjuvant candidates [4,7]. One such candidate is double mutant LT (dmLT); in recent clinical trials it was found that doses up to 100 µg of this adjuvant can safely be administered orally without toxic side effects [8]. While it is hoped that such molecules can be applied to oral vaccines in humans, there is a pressing need for a panel of mucosal adjuvants with the potential to promote humoral and/or cellular immunity against viral and bacterial pathogens. Since no oral adjuvants are currently included in licensed vaccines for humans, the search for alternative, safe and effective, mucosal adjuvant candidates remains an important goal for mucosal vaccine development.

A significant body of adjuvant research has focused on the discovery and development of immunostimulatory compounds

which act directly on antigen-presenting cells (APCs) (e.g. toll like receptor agonists), thus enhancing activation of the adaptive immune system. However, targeting populations of unconventional T cells may represent a highly productive strategy to improve the efficacy of oral vaccines at mucosal surfaces. Mucosal associated invariant T (MAIT) cells are a recently discovered innate populations of unconventional T cells [9,10]. These cells accumulate at mucosal sites, home to mucosal tissue when activated, rapidly produce immunomodulatory cytokines in response to bacterial stimuli, can bridge innate and adaptive immunity, and share common features with the more widely studied invariant Natural Killer T (iNKT) cells, agonists of which have previously been shown to have vaccine adjuvant properties. Together, these characteristics suggest that MAIT cells may be harnessed in the development of oral vaccines.

2. Unconventional T cells: promising targets for future vaccine development

Conventional T cells allow the host to recognize pathogens via the generation of a highly diverse repertoire of receptors specific for antigens presented on major histocompatibility complex (MHC) molecules. These T cell receptors (TCRs) are composed of two peptide chains, with variable (V) and constant (C) domains. The V domain contains three complementarity-determining regions (CDRs) which together form the antigen binding site in the TCR. Conventional T cells expressing an $\alpha\beta$ TCR recognize their specific cognate peptide antigen only when presented by MHC molecules. In contrast to the tremendous diversity in conventional TCRs, a subset of unconventional T cells, characterized by limited T cell diversity exists. These unconventional T cells include $\gamma\delta$ T cells, iNKT cells and MAIT cells [11]. They usually exhibit tissue-specific localization and have “natural memory” phenotype and functions, such as the ability to mount rapid responses following a pathogenic challenge [12]. The most extensively studied unconventional T cells are the iNKT cells and the $\gamma\delta$ T cells [13], both of which have been suggested to have potential immunotherapeutic applications [14–16].

2.1. iNKT cells

NKT cells are characterized by their expression of cell surface markers associated with natural killer (NK) cells and by a TCR, characteristic of T cells. Human iNKT cells have a highly conserved invariant $\alpha\beta$ TCR composed of a V α 24-J α 18/V β 11. They are selected intra-thymically via ligand presentation by the MHC class 1 related CD1d molecule on double positive (DP) hematopoietic cells (CD4⁺ and CD8⁺). NKT cells reside in the intestines of both mice and humans and it is estimated that 1% of intraepithelial lymphocytes (IEL) are iNKT cells in both humans and mice [17], although mice have higher numbers of intestinal iNKTs [18]. iNKT cells express CD161 with variable expression of NK1.1 [19]. A high level of sequence similarity exists between the TCR CDR3 α and CDR2 β loops in human and mouse iNKTs [20]. Expression of a variety of other T cell markers including CD4, but not CD8, has also been reported on iNKTs.

While conventional T cells respond to peptide antigens, the iNKT cells have been shown to respond to glycolipids presented by CD1d [21]. MHC class 1 and CD1 molecules share a common evolutionary origin, however, CD1 molecules are mostly non-polymorphic and have evolved very different antigen-binding grooves which enable them to present hydrophobic lipid molecules [22]. The antigen binding groove of CD1 molecules is defined by two α -helices, which are located above an 8 stranded antiparallel β sheet [23]. Five isotypes of the CD1 molecule family have been identified and these are divided into 2 families, group 1

comprising CD1a–c and group 2 with the sole member CD1d [24]. The fifth CD1d molecule, CD1e has been shown to enhance the loading of antigens into members of group 1. In contrast to other MHC classes, CD1d is essentially monomorphic and can bind to a large selection of self, non-self and synthetic antigens [25]. Both human and murine intestinal epithelial cells (IEC) express CD1d [26], and therefore are capable of activating iNKT cells in the intraepithelial and lamina propria (LP) compartments [27]. CD1d is also expressed on intestinal APCs and B cells. The major ligand classes of CD1d antigens include sphingolipids and glycerolipids. Sphingolipids were the first class of CD1d antigens to be discovered. Glycosylceramides, a subclass of sphingolipids, have been shown to have strong iNKT cell activating potential [28]. The best characterized glycosylceramide, α -Galcer, is derived from a marine sponge *Agelas mauritanus*. The most commonly available form of α -Galcer, KR7000, comprises an α -linked galactose head group and a ceramide base composed of an 28-carbon phyto-sphingosine chain and a 26-carbon acyl chain. α -Galcer has been shown to bind to both murine and human CD1d [28] and the structure of the α -Galcer/CD1d complex has been described [29].

Following antigen presentation, iNKT cells can rapidly secrete both T helper (Th) 1 and Th2 polarizing cytokines [19]. Activated iNKT cells can produce large amounts of interferon (IFN) γ , tumour necrosis factor (TNF) α , interleukin (IL)-4, IL-10, IL-13 and IL-21, and can either promote or suppress cellular responses without the need for clonal expansion (see Fig. 2) [19,30]. Similar to Th17 cells, iNKTs can produce IL-17 and IL-22 (see Fig. 2) [31]. Furthermore, IL-17 producing NKT cells express the Th17 cell transcription factor RAR-related orphan receptor (ROR) γ t and the Th17 cell associated IL-23R. Activated iNKT cells also share NK properties such as the ability to produce granzymes and perforins.

NKT cells were initially found to be involved in tumour immunity, pathogen recognition and autoimmunity [18]. Activation of iNKT cells with α -Galcer was shown to interfere with the induction of oral tolerance [32]. This sparked interest in the application of α -Galcer as a mucosal adjuvant [33,34]. Oral administration of α -Galcer increases migration of lamina propria-dendritic cells (LP-DCs) to mesenteric lymph nodes, which is suggestive of increased CCR7 expression by LP-DCs [35]. Furthermore, LP-DC activation can be enhanced through cytokine production by activated iNKT cells [36]. Depending on the nature of the APC presenting α -Galcer, specific cytokines are secreted by the activated iNKT cell [37], a finding which could be harnessed in novel immunomodulatory strategies. The unique modulatory properties of these cells has driven increasing interest in the therapeutic potential of α -Galcer and other novel iNKT cell activators [16,38]. The immunogenicity of an oral human immunodeficiency virus (HIV) vaccine was strongly enhanced by the inclusion of α -Galcer in the formulation [33].

2.2. $\gamma\delta$ T cells

$\gamma\delta$ T cells develop intra-thymically and are highly evolutionarily conserved between species [39]. These cells have complex functions with roles in both innate and adaptive immunity. They appear in human circulation prenatally and are reported to be the first functional population of circulating T cells. Like iNKT cells they have a more highly restricted TCR V region repertoire than conventional $\alpha\beta$ T cells with TCRs comprising one of 3 V δ chains and at most 6 V γ chains [40]. In adult peripheral blood the majority of $\gamma\delta$ T cells express V δ 2 and V γ 9 chains, while other V γ segments are more abundant in $\gamma\delta$ T cells located in human tissues [39]. Restriction of $\gamma\delta$ T cells occurs independently of MHC and the antigen presenting molecule remains unknown, however, it has been suggested that cell–cell contact is required for activation of these $\gamma\delta$ T cells. They are potentially activated by (E)-4-hydroxy-3-

methyl-but-2-enyl pyrophosphate (HMB-PP), and to a lesser extent by isopentenyl pyrophosphate. Activation results in rapid cytokine production, including IL-17 and IFN γ , and they are also capable of phagocytosis [41].

It has been proposed that $\gamma\delta$ T cells may have an important role in mucosal immunity as well as anti-tumour immunity. Their importance in mucosal immunity is supported by early research which demonstrated impaired production of immunoglobulin A (IgA) in TCR $\delta^{-/-}$ mice following oral immunization with tetanus toxoid and cholera toxin [42].

3. Characteristics of MAIT cells

MAIT cells (referred to as mucosal NKT cells in some earlier literature) are a recently discovered subset of unconventional T cells that are characterized by an evolutionarily conserved invariant $\alpha\beta$ TCR. In humans the invariant TCR consists of a canonical V α 7.2 (TRAV 1–2 genes) chain joined to J α 33 (TRAJ33 gene) which is paired to a limited array of TCR β chains (V β 2, V β 13, or V β 22). Very recently it has been reported that alternative non-J α 33 junctional genes can also be used (including J α 20 and J α 12), and that in combination with this a higher number of β chains can be present [43,44]. In mice the MAIT TCR is composed of V α 19 joined to J α 33, and paired with V β 6 or V β 8 (TRBV19 or TRBV13 respectively). In adult humans, MAIT cells represent a relatively high proportion of circulating T lymphocytes (1–10%), this is at least ten fold higher than circulating iNKT cell numbers [12,45]. However, in cord blood (CB) and infant blood, MAIT cell numbers are very low and are present in a naïve state [12]. Using CD45RO as a marker of memory phenotype, Dusseaux et al. [45] reported an increase in MAIT cell numbers and in memory phenotype, from birth to one–two years of age. By age two, adult levels of circulating MAIT cells were detected [45]. More recently Lee et al. [46] characterized circulating MAIT cells in a large sample of 133 healthy adults and reported high variability in circulating MAIT cell numbers across subjects (0.19–21.7%). Although no significant differences in numbers between males and females were found there was a significant decrease in MAIT cell numbers in elderly subjects ($n = 45$, ages 61–92) versus young subjects ($n = 44$, ages 21–40).

In humans, MAIT cells represent a high proportion of the circulating pool of double negative (DN) lymphocytes (CD4 $^{-}$ and CD8 $^{-}$) and high numbers of MAITs are CD8 $\alpha\beta$ int or CD8 $\alpha\alpha$ + [12,45]. Low numbers of DP and CD4 $^{+}$ MAIT cells have also been reported [12,43,46]. In healthy adults, circulating MAIT cells are in a hypoproliferative state [47,48]. However, this hypoproliferative phenotype can be lost in some inflammatory disease states, for example in inflammatory bowel disease, where there is an increase in circulating activated MAIT cells expressing, Ki67, a marker of cell cycling [48].

MAIT cells also accumulate outside the circulation and owe their name to their preferred localization within mucosal tissues. In paraformaldehyde fixed healthy human jejunal tissue biopsies V α 7.2 positive cells are located in the LP at the base of the villi, and less commonly in the villus tip, and in the IEL compartment [43]. Numbers of MAITs in wild type mice are far lower than in humans and are mainly confined to the intestinal LP and lymph nodes (they were not detected in the liver, bone marrow or spleen in mice) [10,49]. Although initial research demonstrated significant numbers of MAIT cells at mucosal sites, recently, significant accumulation of MAITs was also revealed in the human liver (up to 45% of hepatic T lymphocytes) [45]. These hepatic MAIT cells represent the majority of T cells expressing NK markers and are the dominant IL-17A $^{+}$ T cell subset in human liver sinusoids. Unlike circulating MAIT cells they are reported to express markers of T cell activation,

however, like circulating MAIT cells they have the ability to produce Th1 cytokines and IL-17A [50].

The high basal numbers of MAIT cells in the human intestine makes them an attractive target cell when considering the development of novel oral adjuvants, as they are located directly at the site of vaccine administration, as well as at the site of natural enteric infection. Although iNKT cell activators have shown promising results as vaccine adjuvants [16], the higher distribution of MAIT cells compared to iNKT cells in the humans suggests that targeting MAIT cells may be more successful when developing oral vaccine adjuvants than targeting iNKT cells.

3.1. Cell surface receptor expression

Adult MAIT cells display an effector-memory phenotype (CD45RA⁺ CD45RO⁺ CD27⁺ CD4^{hi} CD95^{hi} CD62L^{lo}), and their chemokine receptor expression pattern (CCR9^{int} CCR7⁺ CCR5^{hi} CXCR6^{hi} CCR6^{hi}) is indicative of preferential homing to tissues, particularly the intestine and the liver [45,49]. Unlike adult peripheral MAIT cells, CB MAIT cells have a naïve phenotype, CD45RA⁺, as do murine MAIT cells [12]. Further profiling of adult MAIT cells indicates surface expression of IL-12r, IL-18r, IL-23r, CD161, NKG2D, ABCB1, α 4 β 7, IL-2R β , and CD26 [51]. The expression of ABCB1 indicates that they have xenobiotic properties as this molecule is responsible for the efflux of toxins from cells. The chemokine receptors and α 4 β 7 expression indicate preferential homing to the mucosal surfaces, including the intestine. The expression of Rorc, CD161, and IL-18r are indicative of Th17-like cell responses and are suggestive of IL-17 producing capabilities. Therefore, based on their G0 status, expression of high levels of ABCB1 expression and ability to secrete IL-17 is consistent with their localization in the gut and liver as they can resist these harsh environments making MAIT cells ideal sentinels [45].

Following *Francisella tularensis* infection, murine MAIT also have a cell surface phenotype indicative of trafficking under inflammatory conditions: positive for CCR2, CXCR3, and CCR5, as well as integrin α 4 β 7. Of these MAIT cells only a small proportion expressed the NK cell markers NK1.1, CD49b, and Ly19d. However, as in the case of human MAIT cells a large proportion were IL-18r α positive (91.1%) [52].

4. MAIT cell development: selection and expansion

A defining paper in characterizing the development of MAIT cells in mice and humans was published by Martin et al. [12]. Prior to this report very little was known about the selection and developmental process; Tilloy et al. [10] had demonstrated the absence of MAITs from nude mice suggesting that the thymus is required for their development, but that expression of major histocompatibility complex (MHC) class I-related (MR1) on epithelial cells was not required for MAIT cell selection. Further analysis of MAIT cell development was hindered by the inability to detect the low MAIT cell numbers in the human or mouse thymus. Later Treiner et al. [53] reported that expression of MR1 on B cells was required for MAIT accumulation in the gut, but again thymic MAIT cells could not be found. Therefore, despite these initial pieces of the developmental puzzle, prior to Martin et al. [12] it was not clear if MAIT cell selection even occurred in the thymus.

In a series of experiments using the recently developed anti-V α 7.2 mAb and transgenic mice it was shown that MAIT cells were indeed present in the thymus [12]. These human intra-thymic MAIT cells were V α 7.2⁺ (3C10⁺), CD161^{hi} and presented with a naïve phenotype (CD45RO^{lo}, CD45RA⁺, and CD27^{hi}), thus excluding the potential for false positive due to peripheral blood contamination. Martin et al. [12] also demonstrated, in foetal thymus organ cultures from V α 19 transgenic mice, that the intra-thymic

selection process is MR1 dependent, and that this is a B cell independent process. However, it was also found that B cells were necessary for expansion and accumulation in the periphery, and that reconstitution of RAG^{−/−} mice with MR1 positive B cells results in peripheral MAIT cell expansion. Together these results demonstrate that MAIT cells use a developmental pathway distinct from other unconventional T cells subsets and conventional T cells, highlighting their position as a unique cell subset with distinctive features [12] (see Fig. 1).

One of the major remaining questions following Martin and colleagues' comprehensive description of the complex developmental process was the hematopoietic cell type responsible for MAIT cell selection in the thymus. Seach et al. [54] determined that MAIT selection requires MR1-expressing CD4⁺CD8⁺ DP thymocytes, and that B cells, macrophages and DCs are all dispensable. The endogenous ligand responsible for this intra-thymic selection by DP thymocytes selection remains unknown.

Although MAIT cells and iNKT cells have many common characteristics, their development is distinct. As discussed above, MAIT cells are selected intra-thymically but develop their memory phenotype extra-thymically, whereas NKT cells are selected, expand and acquire their memory phenotype before exiting the thymus. However, both iNKT and MAIT cells are selected by DP thymocytes (MR1-expressing DP thymocytes for MAIT selection and CD1d-expressing DP thymocytes for iNKT selection).

5. MR1 expression, structure and function

MR1 was originally identified by Hashimoto and co-workers as a cDNA sequence in 1995 [55,56]. MR1 is a non-classical MHC class 1A gene, encoded outside the MHC region on chromosome 1. The MR1 gene is highly conserved (>90% homology) across a number of species including rodents (Mice and Rats) and primates (Humans, Chimpanzees and Orangutans). Tsukamoto et al. [57] attempted to clarify the evolutionary history of MR1 and identify more critically conserved residues for the function of MR1. They carried out a comparative genomic analysis of MR1 using jawed vertebrate genome databases and found that MR1 is only present in placental and marsupial mammals. The MR1 sequence is most highly conserved in the α 1 and α 2 domains between species, with the main amino acid differences occurring in the transmembrane and cytoplasmic domains [56,57]. Based on their high level of conservation, the α 1 and α 2 domains were suggested to shape the ligand-binding groove.

The MR1 protein is a monomorphic, evolutionary conserved MHC class I-like molecule. MR1 transcripts and the intracellular protein are ubiquitously expressed across cells; however, it has been more difficult to confirm cell surface expression of the MR1 protein. This is due to the endogenous cell surface expression levels typically being very low, which is suggestive of a quantitatively limiting antigen being required to increase surface MR1 expression [58]. Although the function of MR1 was unknown until 2003, the possible interaction between MAIT cells and MR1 was hypothesized by a number of authors [10,59]. The possibility of this interaction and broad immunological relevance of MR1 was based on the high amino acid sequence conservation between species, ubiquitous expression of the gene, and evidence of stoichiometric association between MR1 and β 2-microglobulin (β 2m) [59,60]. It was not until knockout mice lacking the MR1 molecule were generated, and shown to completely lack MAIT cells, that it was proven that MAIT cells were selected and/or restricted by MR1 [53]. At approximately the same time the presentation of antigen by MR1 to MAIT cells was also supported by extensive mutagenesis studies [58].

Since the discovery that riboflavin pathway metabolites are ligands for MR1, a number of studies have reported increased MR1

Fig. 1. The pathway of mucosal-associated invariant T (MAIT) cell development, homing to mucosal tissues and activation. Mucosal-associated invariant T (MAIT) cell selection occurs following interaction between MR1-expressing CD4⁺ CD8⁺ double positive (DP) thymocytes and the semi-invariant Va7.2⁺ J α 33T cell receptor. The endogenous ligand required for this selection process remains unknown. MAIT cells have a naïve phenotype (CD45RO^{lo}, CD45RA⁺, and CD27^{hi}) in the thymus (1). Following intra-thymic selection MAIT cells (mainly double negative (DN), CD8 α ^{int} or CD8 α ⁺) migrate to the periphery, where they account for 1–10% of circulating T lymphocytes. In the circulation MAIT cells display an effector–memory phenotype (CD45RA⁺ CD45RO⁺ CD27⁺ CD4^{hi} CD95^{hi} CD62L^{lo}), and their chemokine receptor expression pattern (CCR9^{int} CCR7⁺ CCR5^{hi} CXCR6^{hi} CCR6^{hi}) is indicative of preferential homing to mucosal sites and the liver (2). MAIT cells accumulate at mucosal tissues and in the liver (up to 45% of hepatic T lymphocytes) in humans. Their expansion and accumulation is dependent on the presence of B cells and microbial flora (3). MAIT cell activation occurs via interaction with vitamin B2 metabolites, resulting in the release of cytokines (IFN γ , IL-17, and TNF α), chemokines and Granzyme A and B in a rapid innate type fashion. Activated MAIT cells have cytotoxic potential and have been shown to lyse infected epithelial cells (4).

surface expression following direct incubation of MR1 expressing cells with either the MR1 ligand or bacteria. Kjer-Nielsen et al. [60] report increased upregulation of cell surface MR1 on human lymphoid C1R cells following incubation with these riboflavin pathway ligands. Pre-incubation of DP thymocytes with exogenous bacterial ligand also increases MR1 surface expression and enhances mature MAIT cell activation in *in vitro* co-cultures [54]. It has also recently been demonstrated, in both primary cells and B cell lines, that bacterial infection induces MR1 surface expression, and that this could be induced to a higher extent with certain bacterial strains; most significantly with the commensal *E. coli* strain, BL21, and enteropathogenic *E. coli* [61]. In line with earlier studies, Huang et al. [62] demonstrated that MR1 traffics through endocytic compartments, thereby allowing MAIT cells to sample both endocytosed and endogenous antigens. Furthermore, the stability of MR1 at the cell surface is affected by external factors such as pH [63].

Although multiple isoforms of the MR1 gene are naturally transcribed the majority of research to date has focused on the full length MR1A. Lion et al. [64] have recently shown that a splice variant, MR1B, which lacks the extracellular α 3 domain can be expressed at the cell surface and that its homodimeric surface expression is independent of β 2m. Overexpression of the MR1B transcript is functional as it induced MAIT cell activation *in vitro* in the presence of bacteria [64].

6. MR1 ligands, ligand binding, and MR1-ligand complex binding to MR1

It was originally reported that the α -Galcer-related synthetic glycolipid, α -mannosyl ceramide, activated MAIT cells [65].

However, it proved difficult to reproduce this finding [66], and structural comparisons of the MR1 α/β domain sequences superimposed on the ligand binding platforms of class Ia and Ib molecules predicted that if MR1 does bind to a peptide or a glycolipid, it does so using a unique anchoring scheme not shared with MHC class I or CD1 proteins, respectively [62]. Recently vitamin B2 (riboflavin) pathway metabolites were discovered to act as ligands for the MR1 molecule and induced MAIT cell activation [60]. This discovery distinguishes MAIT cells from peptide or lipid recognizing T cells in the immune system. To date the most potent MAIT cell agonist is the riboflavin pathway metabolite, reduced 6-hydroxy methyl-8-D-ribitylumazine (rRL-6-CH₂OH). The folic acid metabolite, 6-formyl pterin (6FP) also binds to the MR1 molecule; however, it does not induce MAIT cell activation. This finding is consistent with the previous reports that MAIT cells are activated by microorganisms that can synthesize riboflavin.

Recent work conducted by López-Sagaseta et al. [67] and Patel et al. [68] revealed interesting structural, biophysical and biochemical aspects of ligand binding to MR1 and recognition of this complex by MAIT cells. Furthermore, the studies reveal how MAIT cells exert their effector functions via CDR3 β loop diversity and alternate V β gene usage. Crystal structures of the MAIT TCR complex with ligand loaded MR1 molecules revealed that ligands engaging with the CDR3 β loop likely lead to stronger TCR interactions accounting for the enhanced potency of some molecules versus others [67]. However, the diversity of ligand conformations which can bind to MR1 suggests that various types of ligand can be accommodated by MR1, thus increasing the diversity of small molecules that can activate MAIT cells. Moreover, the importance of ligand binding to MR1 is further reinforced by

the increased binding affinity of loaded *versus* unloaded MR1 to MAIT TCR, however the relative contribution of binding affinity to effector function or whether the nature of the MR1 binding ligand can determine MAIT cell specificity remains to be established. The demonstration that MAIT cells can recognize MR1-presented ligands with differential affinities suggests that the TCR diversity generated through CDR3 β loop diversity and alternate V β gene usage may play a role in MAIT cell function [67]. These variations may impart onto MAIT cells the ability to fine tune their immunological response to various MR1 bound antigens allowing for a differential threshold of activation and function. This is especially important when one considers the varied roles of MAIT cells in host responses, both in defence and homeostasis. MAIT cells have been reported to infiltrate into kidney and brain tumours, while also responding to bacterial infections.

Considering their location at the mucosal interface, the ability of MAIT cells to respond to bacterial vitamin metabolites (indicative of an active colonization), makes them ideal sentinels in the GI tract. Sander et al. [69] previously proposed a novel class of pathogen-associated molecular pattern (PAMP), which are

indicative of viable infection, termed viability-associated PAMPs (vita-PAMPs). While Sander et al. [70] focused on bacterial messenger RNA, it has also been speculated that soluble mediators of bacterial metabolism may also act as vita-PAMPs. In the case of MAIT cell agonists these would indeed fall under the category of vita-PAMPs, not just by their biochemical classification as vitamin metabolites but also as indicators of bacterial viability. Thus, the detection of metabolites as opposed to conventional PAMPs and peptide antigens make MAIT cells a more sensitive detector of viable infections at a surface with a high exposure to environmental danger signals.

7. Cytokine and chemokine expression by MAIT cells

As with $\gamma\delta$ T cells and iNKT cells, MAIT cells respond rapidly to ligand presentation by producing immunomodulatory cytokines (see Fig. 2). However, the range of cytokines produced by activated MAIT cells is narrower than activated iNKT cells [43]. Cytokine secretion from human CB MAIT cells has been characterized in supernatants following *in vitro* stimulation with *E. coli* fed

Fig. 2. Cytokine production by conventional and unconventional T cells following TCR stimulation by cognate MHC molecules on APCs. Following stimulation with lipid antigens (e.g. α -Galcer) loaded into CD1d, invariant natural killer T (iNKT) cells produce both Th1 (IFN- γ and IL-17), Th2 (IL-4, IL-13) and Treg (IL-10) like cytokines. Additionally they produce pro-inflammatory cytokines including TNF- α , IL-21 and IL-22. Mucosal associated invariant T (MAIT) cells are activated by vitamin metabolites (e.g. rRL-6-CH₂OH) presented by major histocompatibility complex (MHC) class I-related (MR1). Upon activation MAIT cells produce IFN- γ , TNF- α , IL-2, IL-17 and IL-22, a group of cytokines associated with mucosal immune responses, especially those pertaining to the clearance of bacterial infections. Conventional T cells produce a diverse repertoire of cytokines which is dependent on the nature of secondary signals following stimulation by peptide antigens loaded MHC class I and II molecules on antigen presenting cells (APCs).

monocytes, CD3/CD28 and PMA/Ionomycin [71]. Complete cytokine and chemokine profiling in these cells demonstrated strong responses to *E. coli* fed monocytes but a limited response to anti-CD3/CD28 or PMA/Ionomycin. Stimulation with *E. coli* fed monocytes induced a range of cytokines and chemokines including IL-2, IFN γ , IL-17, and TNF α from CB MAIT cells. However, following stimulation with anti-CD3/CD28, only TNF β secretion was increased. Pre-natal human MAIT cells, from the small intestine, liver, and lung, produced moderate amounts of IFN γ following stimulation *ex vivo* with paraformaldehyde fixed bacteria and CD28 [44,47].

Purified adult MAIT cells appear to be more sensitive to stimulation with anti-CD3 and anti-CD28 or PMA/Ionomycin than CB MAIT cells. Under basal conditions, adult MAITs do not produce any cytokines, and following stimulation do not produce any Th2 (IL-4, IL-5, IL-13) or regulatory T cell associated cytokines (IL-10 or TGF β) but do secrete Granzyme B, TNF α , IFN γ and IL-2. They also produced high levels of IL-17 following stimulation with PMA/Ionomycin but not following stimulation with anti-CD3 and anti-CD28 [45]. As with CB MAIT cells, circulating adult MAIT cells co-cultured with bacteria infected cells (commensal and pathogenic *E. coli* strains) secreted IFN γ , IL-17, and TNF α [61]. Following exposure to *E. coli* fed APCs, Lepore et al. [44] have also reported increases in Th0, Th1 and Th2 cytokines from both J α 33 and J α 12 expressing MAIT cells. Production of Th1 and Th17 related cytokines is not confined to circulating MAIT cells. Increased gene expression and protein concentrations of IL-17A, IL-17F, IFN γ , and IL-2 (see Fig. 2), in response to PMA/Ionomycin, is also seen in human liver MAIT cells [50]. As with circulating MAIT cells, IL-17 is not increased following anti-CD3 and anti-CD28 stimulation in liver MAIT cells, but IL-17 production was higher in response to PMA/ionomycin.

In vivo, MAIT cells have also been shown to be involved in timely production of TNF α , IFN γ , IL-17A, and iNOS transcripts in the lungs of mice infected with *F. tularensis*. This is supported by a lag time in the production of these cytokines in MR1^{-/-} compared with wild type mice [52]. The early production of IFN γ , IL-17 and TNF α *in vivo* is important as these cytokines have significant roles in the generation of the adaptive immune response which is essential for successful vaccination.

The ability of MAIT cells to produce the cytokines, IFN γ and TNF α in response to anti-CD3 and anti-CD28 is dependent on CD161, whereby increased concentrations of anti-CD161 result in a dose dependant decrease in production of these cytokines *in vitro* [51]. This discovery may be of great importance in diseases where there is a decrease in CD161 expressing MAIT cells, such as HIV, and will require further investigation [72,73].

In terms of cytokine production some conflicting observations have been reported in the literature. The production of IL-17 may not be a ubiquitous response from activated MAIT cells and may depend heavily on the type of stimulant, method of MAIT cell sorting, or the subtype of MAIT cell. Dusseaux et al. [45] reported high levels of IL-17 secretion following *in vitro* stimulation with PMA/Ionomycin, with barely detectable levels following stimulation with anti-CD3 and anti-CD28. However, Reantragoon et al. [43] reported no IL-17 production from tetramer sorted MAIT cells following stimulation with any ligand. Failure of MAIT cells to produce IL-17 following exposure to *E. coli* fed APCs was also reported by Lepore and colleagues [44]. This may also be true for IL-4 as, although Lee et al. found that IL-4 was expressed following exposure of MAITs from healthy control PBMCs to PMA/Ionomycin, other studies did not detect any expression of Th2 related cytokines from MAIT cells following stimulation with PMA/Ionomycin or with anti-CD3 and anti-CD28 [45,46,50]. Further research will therefore be required to clarify some of the differences in cytokine expression reported.

8. Potential problems with the classification and/or characterization of MAIT cells to date

Birkinshaw et al. [72] very recently reviewed the advances that have been made in MAIT cell research. They highlighted a number of potential problems that may have been overlooked in past analysis of MAIT cells and demonstrated the increased availability of tools which should allow for future clarification. Firstly, the majority of research to date classifies human MAIT cells as CD3⁺, CD161^{hi}, and V α 7.2⁺ which has allowed their function and location within the circulation and tissues to be characterized. However, it should be noted that as this classification strategy relies on reactivity with the anti-V α 7.2 mAb, and because this is not fully limited to MAIT cells, some misinterpretations may have been made in the past. The recent development of an MR1-tetramer should resolve any of these issues [43]. Secondly, this current strategy also relies heavily on the high expression of CD161 and thus any decreases in its expression under varying conditions may result in false negatives when measuring MAIT cell numbers. A good example of such an occurrence is the possibility that the decrease in circulating MAITs reported in HIV patients may in fact reflect a decrease in CD161 expression in the existing MAIT cells rather than an actual decrease in cell number. This characteristic may still be very important in these disorders, as CD161 modulates the cytokine response triggered by bacterially infected cells [51].

Another major difficulty when studying MAIT cells is their very low levels under basal conditions in wild type mice which makes it very difficult to study their physiological behaviour *in vivo*. This problem is further compounded by the lack of an anti-V α 19 mAb, making the analysis process more open to error due to a two step process being required; initial sorting of cells is carried out using flow cytometry and the V α 19 expression is analyzed by PCR [52]. Again the recent availability of the MR1 tetramer should allow for rapid advancements in the specific analysis of mouse MAIT cells.

9. Antibacterial properties of MAIT cells

The absence of peripheral MAIT cells in germ free mice provided the first evidence of a close relationship between MAIT cells and bacteria. Interestingly, the requirement for direct exposure to commensal microflora does not appear to be the case in humans as prenatal development of mature MAIT cells and their enrichment in the lungs and intestines is seen. This is prior to establishment of intestinal microflora or exposure to environmental bacteria [47]. It is possible, however, that small molecule exogenous ligands from bacteria may be transferred from the mother to the foetus resulting in MAIT cell development before postnatal bacterial colonization. This would still indicate that a relationship between MAIT cells and bacteria is essential for human MAIT cell development and will require further analysis.

It is now clear based on a number of *in vitro* and *in vivo* studies that MAIT cells are involved in combating bacterial infections via a number of mechanisms including cytokine secretion, recruitment of conventional T cells, and lysis of MR1-expressing bacterially infected cells [44,52,74,75]. MAIT cells display a clear antibacterial function which may be attributed to their production of IFN γ , however, as the levels of IFN γ are less than that of conventional T cells, additional antibacterial activity may be due to their ability to recruit IFN γ producing conventional T cells [52]. As mucosal tissues represent the entry sites of the majority of pathogens, the accumulation of MAIT cells in these tissues along with their homing abilities to mucosal tissues confers them with an additional antibacterial advantage. Although human MAIT cell development has recently been suggested to be distinct from the previously proposed mouse MAIT cell process [47], pre-natal MAIT cells have been shown to display antimicrobial responsiveness,

therefore, this is consistent with their previously described antibacterial function. Thus, MAIT cells may provide early protection to newborns from bacterial infections, during a time where the adaptive immune system is immature. This prenatal establishment of MAIT cells further supports the idea that they form an early barrier to infection at mucosal surfaces, while the T cell compartment is still undergoing development.

9.1. In vitro activation of MAIT cells

In vitro, transgenic mouse MAIT cells (iV α 19-V β 6 and iV α 19 transgenic mice) and human MAIT cells respond to bacteria and yeast in an MR1 dependent manner (see Fig. 1). Co-culture of MAIT cells with bacteria infected bone marrow-derived DCs shows they are activated by a range of gram-negative and gram-positive bacteria including *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Lactobacillus acidophilus*, *Staphylococcus aureus*, and *Staphylococcus epidermidis* [51]. However, they are unresponsive to certain bacterial classes (*Enterococcus faecalis* and *Streptococcus* group A), that do not have the ability to metabolize vitamin B2 (riboflavin). In similar co-cultures, MAIT cells also responded to a range of yeast infections including *Saccharomyces cerevisiae*, *Candida glabrata* and *Candida albicans*, however, they were unresponsive to any viruses including Sendai virus, Newcastle disease virus, herpes simplex virus and parainfluenza 3 virus [51]. This ability of MAIT cells to respond to infected cells relies on direct cell-cell contact between MAIT cells and the APC [61].

Not only can MAIT cells detect bacteria and produce cytokines/chemokines in a rapid innate type fashion but they also have the capability of lysing infected cells, making them the most abundant T cell subset able to detect and kill bacteria infected cells in humans [51]. Consistent with their expression of CD8 co-receptors and ability to secrete Granzyme A and B following TCR ligation, Le Bourhis et al. [51] demonstrate that in response to non-invasive *E. coli* infected epithelial cell lines overexpressing hMR1, MAIT cells were cytotoxic to these infected cells in an MR1 dependent manner. Functionally, this characteristic might be of critical importance to their mucosal localization, as bacterial infection of epithelial cells represents an initial signal that normal barrier function may be under threat. This ability to respond to bacterial metabolites and to kill infected cells may, therefore, protect the body from dangerous bacterial translocation beyond the confines of the mucosal surface.

9.2. Antibacterial activity of MAIT cells in vivo

The lack of MAIT cells in germ free mice, and the finding that reconstitution with single bacterial strains induces MAIT cell population expansion points to an antibacterial function [76]. However, when wild type mice and MR1^{-/-} mice, were compared in terms of ability to fight *Mycobacterium abscessus* (I.V.), *Echerichia coli* (I.P.), *Shigella dysenteriae* (I.P.), or *Yerinia enterocolitica* (I.P.) infection, no significant differences in bacterial susceptibility were seen [75,77]. On the contrary, with infections such as *Klebsiella* (I.P.) infection, increased bacterial counts at day 10, increased bacterial spread to the lung, altered cytokine profile (decreased IL-1 β , TNF α and IL-17), and increased mortality rates were found in MR1^{-/-} compared to WT mice [77].

Chua et al. [78] propose two explanations for the apparent discordance between the reported antibacterial effects of MAIT cells *in vitro* and *in vivo*. Firstly, the low numbers of MAIT cells in WT mice may not be sufficient to allow for antibacterial clearance. This is supported by evidence of decreased bacterial colonization in MR1⁺ compared to MR1^{-/-} iV α 19-V β 6 and iV α 19-transgenic mice [51]. Secondly, due to the mucosal localization of MAIT cells and mucosal homing abilities their antibacterial function might only be

productively exploited for mucosal infections. In support of this hypothesis WT mice infected mucosally (via aerosol or nasal route) with *Mycobacterium bovis* Bacillus Calmette-Guérin (BCG), exhibited decreased bacterial loads at early time points post infection compared to MR1^{-/-} mice [78].

Although the response of MAIT cells to enteric bacterial infection has not been addressed, their response to pulmonary infection has been described [52]. In response to a sub-lethal nasal dose of the live vaccine strain (LVS) of *F. tularensis*, DN T cells appeared early following infection and numbers peaked in the lungs of mice at day 14 post infection. This was associated with increased V α 19-J α 33 gene expression suggesting that these cells were MAIT cells. This increase in DN cells was not observed in MR1^{-/-} mice supporting the proposal that these DN cells in WT mice were in fact MAIT cells. Consistent with the reported chemokine receptor expression pattern on MAIT cells, which is indicative of preferential homing to mucosal tissues, Meierovics et al. reported preferential accumulation of MAIT cells in the lungs following pulmonary infection, with little or no accumulation in spleens, mediastinal lymph nodes, or mesenteric lymph nodes [52]. Accumulation of MAIT cells in the lungs was essential for optimal resistance to *F. tularensis* infection as well as early production of antibacterial cytokines. MR1^{-/-} mice exhibited a significantly higher bacterial load at 10 days post infection and slower release of pro-inflammatory cytokines and iNOS, which are critical antimicrobial factors. MAIT cells were also essential for the timely recruitment of activated IFN γ producing CD4⁺ and CD8⁺ T cells during *F. tularensis* infection which is essential for the elimination of the bacteria. Overall, CD4⁺ and CD8⁺ T cells were shown to be necessary for the complete clearance of the infection as WT mice depleted of CD4⁺ and CD8⁺ T cells developed a chronic infection but all mice survived up to 2 months post infection. In contrast, MR1^{-/-} mice depleted of CD4⁺ and CD8⁺ T cells did not survive beyond 40 days post infection. Together this *in vivo* data shows a non-redundant effect of MAIT cells in controlling mucosal bacterial load following pulmonary infection, and that their activation influences both the innate and adaptive phases of mucosal immunity.

9.3. Antibacterial activity of MAIT cells in vivo in humans

In humans evidence of the close association between bacterial infection and MAIT cells was initially shown in patients suffering from pulmonary bacterial infections [76,79]. Decreased circulating numbers of MAIT cells were observed in patients suffering from tuberculosis (TB) compared to healthy controls and cancer patients. Two potential explanations for the decrease have been suggested: either the circulating MAIT cells succumb to apoptotic cell death, or homing to the site of infection occurs. Increased MAIT cells in lung lesions of two patients with active TB, suggests that migration to the infected tissue is the cause of the decreased circulating numbers. In a separate study, *ex vivo* analysis also demonstrated that mycobacterium tuberculosis (Mtb)-reactive MAIT cells were enriched in the lungs of patients with active TB, and responded to Mtb-infected MR1-expressing lung epithelial cells [79].

Despite no rodent studies to date examining the implications of enteric bacterial infection on MAIT cell numbers or their activation state, MAIT cell activation has been suggested following enteric bacterial infection in humans. In volunteers receiving an oral dose of attenuated *Shigella dysenteriae*, circulating MAIT cell numbers were decreased at day 11 post vaccination when compared to a placebo control group. In volunteers that responded to the vaccine by producing antigen-specific IgA levels, above the threshold of 20 antibody secreting cells per 10⁶ peripheral blood mononuclear cell, MAIT cells were also more activated than in the non-responders at this time-point [51].

The importance of MAIT cells in bacterial infection has been further confirmed in a large clinical study involving patients

suffering from severe sepsis or septic shock [80]. A comparison of patients suffering from severe bacterial infections ($n = 113$), no infection ($n = 36$) or severe viral infections ($n = 7$), compared with healthy controls ($n = 26$) revealed that a marked decrease in circulating MAIT cells occurs early following bacterial infection. In contrast to MAIT cell depletion, no difference in numbers of other innate T cell types (iNKT or $\gamma\delta$ T cells), was found in these patients. Importantly persistent MAIT cell depletion was associated with increased risk of secondary intensive care unit (ICU)-acquired infections. Therefore, in addition to providing evidence supporting the antibacterial role of MAIT cells in humans, this study suggests that circulating MAIT cell numbers may be a useful biomarker for risk of developing ICU-acquired infection. Non-streptococcal bacteria were identified as the most significant independent predictor of decreased MAIT cell count in patients. This correlates with previous research in mice which also demonstrates a larger decrease in MAIT cell counts in mice infected with non-streptococcal versus streptococcal bacteria [76].

10. Conclusions: MAIT cells as novel vaccine targets

MAIT cells have the potential to bridge the innate and adaptive arms of the immune system and play a key role in immunity to bacteria. Based on the immunomodulatory potential of MAIT cells and their mucosal localization, specific agonists may represent novel mucosal adjuvants. As no oral adjuvants are currently licenced for human use, this could be very useful in improving the

immunogenicity of oral vaccines. The localization of MAIT cells in the gut and lung is particularly relevant for mucosal vaccination as these sites represent prime locations for pathogen attack but also immunization. Secondly, MAIT cells can respond rapidly to bacterial infection. Their activation results in the production of chemokines and cytokines which can lead to the recruitment of other immune cells, including DCs that can promote antigen specific immunity.

Although it has previously been suggested that MAIT cells could be a target for novel vaccines to date, no studies have directly examined the adjuvanticity of MAIT cell agonists [52,72,75,76]. In contrast to NKT cells, direct evidence that MAIT cell activation results in enhanced humoral or cellular adaptive immunity remains to be provided. The recent discovery that riboflavin pathway metabolites can activate MAIT cells [60] should allow for future experiments to examine the mucosal adjuvanticity of these agonists. Beyond determining the oral adjuvanticity and safety profile of MAIT cell agonists *in vivo*, it will be essential to determine if these ligands are sufficiently robust to overcome the harsh acidic gastric environment and resist proteolytic degradation in the duodenum. Furthermore, their ability to drive local responses in regions of MAIT cell abundance will be critical. It should be mentioned that if MAIT cell agonists do not satisfy these criteria in their native form that a suitable delivery system should be employed in order to facilitate the transit of these molecules not only to the right anatomical location in the GIT but also to ensure that they reach the location intact (see Fig. 3). Current oral delivery

Fig. 3. Vaccine delivery route and methods for enteric formulations containing mucosal-associated invariant T (MAIT) cell agonists. Overcoming the challenges faced by enteric vaccines is key to developing viable prototypes. While both the nasal/bronchial and oral routes have to contend with mucus membranes, vaccines administered orally also have to contend with the acidic environment of the stomach, intestinal digestive enzymes and an area of immune hypo-responsiveness predisposed towards tolerance. While activating MAIT cells via their agonists may prove to be a novel strategy for improving the immunogenicity of novel oral vaccine formulations, delivering these through regions of the gastrointestinal tract (GIT) where they face possible destruction together with ensuring their release at the optimal anatomical location will be key. (1) Using nanoparticles it is possible to conjugate antigens (Squares) and MAIT cell agonists (Triangles) to particles ensuring their joint delivery to cells. Further targeting specificity can be afforded using antibodies and lectins specific for cell-surface markers. (2) Capsular delivery is advantageous as it is possible to protect formulations, as they pass through the stomach, via a pH-sensitive enteric coating. Furthermore, by varying the composition of this coating it is possible to determine the location of release in the GIT. Uptake of antigens and agonists at the site of release can also be influenced by the choice of capsule materials such as exterior shell and interior components. (3) Liposomes present another delivery option where vaccine components are surrounded by a lipid bilayer. By varying lipid composition and charge it is possible to enhance uptake of these in the GIT. (4) Nasal sprays involve the use of a suitable solution to deliver vaccine components to target cells. Combined delivery of antigens to APCs and MAIT cell agonists to MR1 expressing cells could greatly improve the efficacy of mucosal vaccines with the overall outcomes being an enhancement of both local mucosal antibody responses and systemic immunity to enteric pathogens.

systems, including nanoparticulate carriers, oil-in-water emulsions, capsules and liposomes could be helpful in improving the efficiency of MAIT cell agonists.

The most potent activator of MAIT cells, rRL-6-CH₂OH, is currently unavailable commercially, however, additional insights into the binding of riboflavin pathway metabolites to MR1 should lead to the generation of synthetic mimetics which can emulate the natural ability of these molecules to stimulate MAIT cell activation at a lower production cost with greater control over the molecule's adjuvanticity. One other exciting prospect is that bacteria could be engineered to over produce a MAIT cell agonist such as rRL-6-CH₂OH, thus allowing for live attenuated bacterial vaccine strains to produce their own adjuvant. This proof of concept has been realized in *Salmonella* vaccine strains engineered to over produce the $\gamma\delta$ T cell activator HMB-PP [81], and could offer an alternative solution to the chemical synthesis method required for rRL-6-CH₂OH production.

Despite this combination of localization and function being suggestive that MAIT cells are a potential target for the development of oral vaccine adjuvants, age should be considered if pursuing their use. Enteric infections are most fatal during the first years of life, however, this is also a time when MAIT cell counts are at their lowest. Therefore, the potential benefits of MAIT cell ligands in bridging the innate and adaptive immune response may not be as effective in very young children most in need of protection. It has also been suggested that a shift in cytokine response is seen when MAIT cells from older and younger adults are compared. The possible influence of age on Th1/Th2 cytokine response may also be fundamental in predicting the response to a vaccine and requires further investigation. However, based on the available evidence, strategies may be developed in the near future to effectively engage gastrointestinal MAIT cells, thus improving the efficacy of existing oral vaccines and promoting the development of prototypes which in time will help alleviate the global burden of enteric infections.

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