Review Article

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T cell recognition of non-peptidic antigens in infectious diseases

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The immune system has evolved to recognize a wide range of antigenic molecules of self and non-self origin. The stimulatory antigens form complexes with antigen-presenting molecules and directly interact with the T cell receptor (TCR). Peptidic antigens associate with major histocompatibility complex (MHC) molecules and therefore, are indicated as MHC-restricted. Non-peptidic antigens do not bind to MHC molecules and are presented by other classes of antigen-presenting molecules. These non-MHC restricted antigens include glycolipid molecules, phosphorylated metabolites of the mevalonate pathway and vitamin B2 precursors. T cells specific for non-peptidic antigens have important roles in host defense against infections, autoimmunity, allergies and tumour immunosurveillance. Hence, understanding the molecular interactions between the antigen presenting cell (APC) and the T cells with non-peptidic specificity is of great relevance. Here, we review current knowledge of this type of T cells, their TCR repertoire, the structural aspects of recognized antigens, the mode of antigen recognition, and their function with special emphasis on their role in infectious diseases.

Key words Microbial infection - non-peptidic antigens - peptidic antigens - T cell - T cell receptor - TCR repertoire - Toll like receptors

Introduction

Non-MHC (major histocompatibility complex) restricted T cells generally use less diverse T cell receptor (TCR) repertoire compared to the conventional MHC-restricted T cells and their TCRs are highly conserved among different species[1,2]. Based on the restriction molecule, antigen specificity and TCR structure, non-MHC restricted T cells are grouped into three categories: lipid-specific T cells, gamma delta (γδ) mucosal associated invariant T cells (MAIT).

Lipid-specific T cells

Lipid-specific and peptide-specific T cells share similar cell lineage and immunological functions but different antigen presentation and thymic selection mechanisms[3]. A large number of lipid-specific T cells has been associated with immunoregulation as well as protection during infections. Lipid antigens are recognized by the TCR as complexes formed with the CD1 antigen-presenting molecules.

Structure of CD1 molecules

The human CD1 family consists of five glycoproteins, which can be classified on the basis of their sequence similarity as follows: group 1 consists of CD1a, CD1b, and CD1c; group 2 consists of CD1d; CD1e is classified into a third separate group, due to its unique structural features. In mice and rats, only CD1d is expressed. These CD1 molecules are expressed by professional antigen presenting cells (APCs) and CD1-lipid antigen complexes are formed within cellular compartments where CD1 molecules recycle.

All CD1 molecules show structural features similar to MHC class I molecules, including two α helices in the distal domains and one α3 domain that non-covalently associates with β-2 microglobulin. CD1 molecules have an antigen-binding groove consisting of deep and narrow hydrophobic pockets[4-8]. The hydrophobic amino acids surrounding the pockets facilitate anchoring of the aliphatic moieties of lipid antigens, thus protecting these from aqueous surrounding. CD1e molecules never reach cell surface and are involved in processing, loading and unloading of glycolipid antigens[7,9]. Therefore, CD1e is not a *bona fide* antigen-presenting molecule, however, it contributes to important aspects of lipid antigen presentation.

Restriction and TCR repertoire of lipid-specific T cells

Based on the restriction pattern lipid-specific T cells can be divided into two groups: those that are restricted by group 1 CD1 molecules, having similar properties to peptide-specific T cells, and those that are restricted by CD1d molecules[3,10,11]. In humans, group 1 CD1-restricted T cells use diverse αβ TCRs, similar to MHC-restricted αβ T cells. Till date the size and repertoire of the group 1 CD1-restricted T cell pool remain poorly understood. In the blood of normal donors large numbers of CD1a-autoreactive T cells can be found that express the cutaneous lymphocyte antigen (CLA), a homing receptor that identifies T cells participating in skin immune responses and produce IL-22[12]. In a parallel study, it was found that up to 10 per cent of circulating T cells were CD1a- and CD1c-autoreactive[13]. These latter cells express a polyclonal TCR, are naïve at birth, increase in number with age, and acquire the phenotype of memory cells. These findings suggest maturation and expansion dynamics that resemble those of classical, MHC-restricted T cells. The antigen/s stimulating this population of autoreactive CD1-restricted T cells remains unknown.

CD1d-restricted T cells co-express natural killer (NK) cell markers and are known as natural killer T (NKT) cells. Based on the utilization of TCR α and β genes two major subsets of NKT cells have been described in mice and humans. Type I or invariant NKT (iNKT) cells express a semi-invariant Vα14Jα18 TCRα chain in mice and Vα24Jα18 TCRα chain in humans combined with a limited set of TCRα chains (Vβ7, Vβ8, and β2 in mice and Vβ11 in humans)[14]. Available evidence suggests that the β chain of iNKT TCR is important in recognition of different lipid antigens[15] and shapes the functionality of iNKT cell repertoire[16]. In contrast, type II or diverse NKT (dNKT) cells express diverse TCRα and β chains. The TCR diversity of dNKT cells suggests distinct antigen specificities, phenotype, and function from those of iNKT cells[17]. dNKT cells use features of both innate-like and conventional T cells during antigen recognition[18,19]. Both type I and type II NKT populations are conserved in mice and humans, and can express either CD4 or CD8 or be CD4-CD8 double negative, with the exception of mouse type I NKT cells that are never CD8 positive[14,20]. Type I NKT cells are more abundant in mice (approximately 2.5% of mouse T cells in the spleen and lymph nodes, and 30 % of the T cells in the liver) and type II NKT cells in humans where these constitute a significant proportion of the T cells in bone marrow, liver, and gut[14,21,22]. Moreover, type II NKT cells have been recently shown to display distinct but overlapping antigen specificities for different lipid antigens, indicating foreign-lipid reactive repertoire[17].

Lipid-antigen structure and properties

Unlike water-soluble peptides, lipid molecules are not soluble in water and are always associated with either lipid-binding-proteins or membranes in tissues and biological fluids, making their biology and immunogenicity altogether different from those of peptides. Several lipid antigens of bacterial origin have been characterized up to now (Table)[17,23-28]. *Mycobacterium tuberculosis*-derived mycolic acid (MA) is the first lipid antigen that was shown to stimulate CD1-specific T cells[25]. MA is CD1b-restricted and has a α-branched β-hydroxy long chain fatty acid in which the hydroxyl (-OH) and carboxyl (-COOH) groups are recognized by T cells. MA attached to a single glucose molecule forms glucose monomycolate (GMM), another mycobacteria derived CD1b-restricted immunogenic lipid. As GMM is synthesized by pathogenic mycobacteria upon utilization of glucose derived from its host, it has been

Table. Microbial lipid antigens presented by CD1 molecules

Antigen	Origin	Restriction	References
Didehydroxymycobactin	*Mycobacterim tuberculosis*	CD1a	23
Lipoarabinomannan	*M. tuberculosis*	CD1b	24
Lipomannan	*M. tuberculosis*	CD1b	24
Phosphatidylinositol mannoside	*M. tuberculosis*	CD1b	24
Mycolic acid	*M. tuberculosis*	CD1b	25
Glucose mono mycolate	*M. tuberculosis*	CD1b	24
Glycerol mono mycolate	Mycobacteria, *Nocardia,* Corynebacteria	CD1b	26
Diacylsulphoglycolipid	*M. tuberculosis*	CD1b	27
Mannosyl-β-1-phosphomycoketide	*M. tuberculosis*	CD1c	28,29
C32-Phosphomycoketide	*M. tuberculosis*	CD1c	30
α-Galactosylceramide	*A. mauritianus*	CD1d	31
α-Galacturonosylceramide and α-Galactosylceramide	*Sphingomonas* spp. *Novosphingobium aromaticivorans*	CD1d	32,33,34
α-Galactosyldiacylglycerol	*Borrelia burgdorferi*	Cd1d	35
Phosphoatidylinositol-tetramannoside	*M. tuberculosis*	CD1d	36
Cholesteryl α-glucoside	*Helicobacter pylori*	CD1d	37
Lysophospholipids	Bacteria	CD1d	38
Phosphatidylglycerol	*M. tuberculosis* *Corynebacterium glutamicum*	CD1d	17
Diphosphatidylglycerol	*M. tuberculosis* *Corynebacterium glutamicum*	CD1d	17
Phosphatidylinositol	*M. tuberculosis* *Corynebacterium glutamicum*	CD1d	17

suggested that the immune response against GMM might distinguish pathogenic from non-pathogenic mycobacteria[39]. Other immunogenic mycobacterial CD1b-restricted lipids are glycerol monomycolate (GroMM), phosphatidylinositol mannoside (PIM), lipoarabinomannan (LAM)[24], lipomannan (LM)[24], and diacylsulphoglycolipid (Ac_2SGL)[26,27,39]. Mannosyl-β-1-phosphomycoketide (MPM), another mycobacterial lipid antigen with one lipid tail[28,29] is stimulatory in association with CD1c and its tail is composed of a single saturated alkane with methyl branches on every fourth carbon. Recently, using an organism-wide survey, C32-phosphomycoketide (PM) from *M. tuberculosis* has been identified as another CD1c-restricted lipid antigen[30].

Also iNKT cells recognize lipid antigens from several microorganisms. These include α-galactosylceramide (α-GalCer) from *Agelas mauritianus*, the first iNKT antigen[31]; α-glucuronosylceramide and α-galacturonosylceramide from *Sphingomonas* species[32,33]; cholesteryl α-glucoside from *Helicobacter pylori*[37]; α-galactosyldiacylglycerol from *Borrelia burgdorferi*[35]; tetramannosylated phosphatidylinositol (PIM_4) from *M. bovis*[36]; and lipophosphoglycan from *Leishmania donovani*[40]. In contrast, dNKT cells do not recognize the α-glycan-linked microbial lipid antigens that stimulate iNKT cells and it remains to be investigated whether microbial glycolipid antigens stimulate this type of cells. On the contrary, lysophospholipids from mycobacteria and Corynebacteria stimulate dNKT hybridomas in a CD1d-restricted manner[16]. Interestingly, these dNKT hybridomas showed cross-reactivity to self-phospholipids with structures similar to those of microbial antigens, raising the issue of whether self-phospholipid antigens are involved in positive selection, tolerance and eventual role in autoimmunity of dNKT cells.

Effector functions of CD1-restricted T cells

Following primary stimulation iNKT cells acquire an effector / memory phenotype and rapidly secrete large amounts of Th1 and Th2 cytokines[14]. Fast secretion of cytokines is controlled by the strength of TCR signal received during antigen recognition[41],

which depends on the number of CD1 complexes as well as their persistence on the surface of APCs. These latter parameters are in turn influenced by CD1e[9]. The effector capacities of iNKT cells contribute to the activation of other cells including dendritic cells (DCs), NK cells and other lymphocytes, and thus iNKT cells can be considered as important regulatory cells of adaptive immune responses. Further, iNKT cells express a variety of chemokine receptors that may drive their traffic to inflamed tissues[42]. Intriguingly, iNKT cells display poor expression of the lymphoid homing receptors CCR7 and CXCR5, suggesting that their function is mostly exerted in peripheral tissues rather than in lymphoid organs.

The function of group 1 CD1-restricted T cells is different from that of iNKT cells. These have been indicated as important cells in the immune reaction during bacterial infections. Their migratory capacity is less clear, due to the difficulty in generating appropriate reagents, although these can migrate to both lymphoid organs and peripheral tissues. Their priming occurs after infection as shown by experiments conducted in humans, in which CD1b-restricted T cells expand following bacillus Calmette-Guérin (BCG) immunization and *M. tuberculosis* infection[27,43]. Furthermore, immunization of guinea pigs with *M. tuberculosis* lipids, which have CD1b, protects them from subsequent challenge[44]. This protection was associated with the generation of CD1b-restricted response in vaccinated animals.

CD1-restricted T cells in infections

Lipid-specific T cells are important participants in human immune responses and recognition of lipid antigens contributes to host defense against a variety of pathogens, including mycobacteria, viruses, and parasites. MA-specific CD1b restricted T cells have been detected in the periphery and lungs of tuberculosis (TB) patients[45]. These T cells produce interferon (IFN)-γ and interleukin (IL)-2, exhibited effector and central memory phenotypes and were absent in uninfected BCG-vaccinated controls. MA-specific responses contracted markedly with the declining pathogen burden in patients following treatment. Furthermore, these MA-specific cells exhibited recall expansion upon antigen reencounter *in vitro* long after successful treatment, indicating persistence of lipid-specific memory T cells. These findings suggest that mycobacterial lipids may be promising targets for improved TB vaccines.

Infections with bacteria modulate the expression of CD1 molecules and CD1-restricted responses. *M. tuberculosis* downregulates the expression of CD1 molecules in the infected monocytes and dendritic cells (DCs), leading to their reduced presentation of lipid antigens[46-48]. The mechanisms of downregulation of CD1-restricted presentation by *M. tuberculosis* remain to be investigated. Several viruses, including HIV-1, herpes simplex virus (HSV) 1 and human cytomegalovirus (CMV), downregulate CD1d expression and have inhibitory effects on CD1d antigen presentation[49-51]. In contrast, *Listeria monocytogenes* infection upregulates CD1d expression contributing to iNKT cell activation[52]. Recently, it was shown that iNKT cells produce IL-22 during influenza A virus infection and protect against lung immunopathology[53]. iNKT cell activation may lead to the production of IFN-γ and to activation of bystander cells including DCs, NK and cytotoxic cells. These multiple mechanisms contribute to protection against pathogens.

Gamma-delta (γδ) T cells

In humans a major population of T cells expresses the TCR γδ. Indeed, γδ T cells constitute about 5 per cent of circulating CD3-positive cells and are most often CD4-CD8 double negative or CD8 positive[54]. Despite γδ T cells represent a small population when compared to αβ T cells, these show peculiar functional characteristics that make these unique and important.

The repertoire of TCR γδ cells

The TCR γ locus maps to chromosome 7 and spans 160 kb of DNA in the human genome. The γ locus comprises two constant gene segments (Cγ1 and Cγ2) and five joining elements, J1, JP, and JP1 located upstream of Cγ1 and JP2 J2, upstream of Cγ2. Six in frame variable γ (Vγ) genes have been identified[55]. These genes are classified into four subgroups (VγI-VγIV), with five functional genes (Vγ2, 3, 4, 5, 8) belonging to the VγI family. Family II consists of only one gene, Vγ9 (designated Vγ2 in another nomenclature). Cγ1 and Cγ2 genes may give rise to different γ chains. Exon 2 of Cγ1 encodes the cysteine residue forming a disulphide bridge with the δ chain, and thus the γδ TCRs using Cγ1 are disulphide linked. Cγ2 has an allelic polymorphism with either 2 or 3 copies of an exon homologous to Cγ1 exon 2 but without codons for this cysteine residue[56]. Thus, γδ TCRs using Cγ2 are not disulphide linked to the δ chain.

The human δ locus is on chromosome 14 within the TCRα locus. One Cδ gene segment is located in

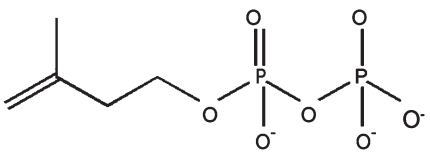
front of the Jα segment cluster and is preceded by four different Jδ segments. Three diversity (D) elements are also identified in front of the Jδ cluster[56]. Only six Vδ chains are expressed by human γδ T cells[57]. Vδ2 is the most 3' Vδ gene, followed in a 3'-5' direction by Vδ 8, 7, 5, 1, 6 and 4. Instead the Vδ3 gene is located at 3' of Cδ[58]. The junctional diversity of the rearranged δ genes is much greater than that of other TCR genes as up to three Dδ segments can be used in tandem, together with imprecise joining and extensive incorporation of N nucleotides[59]. These characteristics generate TCR δ chains with an extremely high variability in the CDR3 region, which may encode a potential TCR γδ repertoire at least three orders of magnitude larger than the TCR αβ repertoire. This property may have important implications in the antigen recognition by γδ T cells.

Another striking feature of the TCR γδ is the preferential association of Vδ and Vγ chains. Vδ2 mostly associates with Vγ9 chain, while Vδ1 and Vδ3 chains mostly associate with Vγ chains other than Vγ9. This preferential association seems to be generated by some kind of antigen selection, since mixed combinations are found in γδ T cell clones isolated from thymus[60]. A recent study that used deep sequencing of TCR γ genes in T cells from peripheral blood showed a striking oligoclonality, as 25 clonotypes accounted for almost 10 per cent of total TCR γ repertoire[61]. Interestingly, when TCR αβ cells were subjected to the same analysis, there was a large number of out of-frame TCR γ sequences in all tested individuals, indicating that TCR γδ cells had been rearranging the TCR γδ locus before becoming committed to the TCR αβ lineage[61].

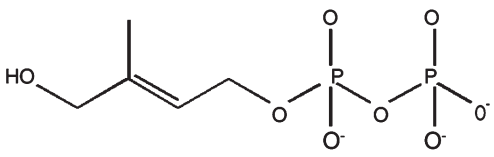
Antigens stimulating TCR γδ cells

The major population of human γδ T cells that expresses the TCR Vγ9Vδ2 recognizes small phosphorylated metabolites. These molecules are isopentenylpyrophosphate (IPP) generated in the mevalonate pathway[62,63], and 4-hydoxy-3-methyl-but-2-enyl pyrophosphate (HMBPP) that is an intermediate metabolite of the 2-C-methyl-D-erythritol 4-phosphate biosynthetic pathway present in several microorganisms[64] (Fig. 1). These antigens closely resemble each other, although HMBPP shows 1000 times higher potency than IPP when added exogenously to APCs. The fact that the microbial metabolite is very potent supports the hypothesis that this major T cell population is involved in anti-microbial surveillance. Comparison of the potency of different synthetic analogs of IPP has shown that the length and structure of the alkyl chain are important for immunogenicity. Indeed, methylphosphate is active, whereas dimethylphosphate and trimethylphosphate are not stimulatory[65]. Also, the number and position of the phosphate groups play a critical role[66]. An important finding was that drugs capable of inhibiting the mevalonate pathway also controlled the activation of γδ T cells. Aminobisphosphonates stimulate Vγ9Vδ2 cells by inhibiting the late steps of the mevalonate pathway, and inducing the accumulation of IPP, an intermediate metabolite[67]. The mechanism how these antigens interact with TCR γδ remains unclear. Studies have indicated that APCs are important, and that IPP and HMBPP require presentation by dedicated antigen-presenting molecules, which are non-polymorphic, ubiquitous and species-specific[68-71].

The other population of γδ T cells recognize different types of antigens, which are different from the stimulatory phosphorylated metabolites. However, in most of the cases only individual T cell clones have been identified with defined specificities, raising the issue of whether these are unique cases not representing the majority of other γδ cells. Some γδ cells showed alloreactivity against MHC molecules[72,73], or were activated by CD1c-[74,75], CD1d-[76], and CD48-expressing cells[77]. More recently, the reactivity of one Vδ1T cell clone against the endothelial protein C receptor (EPCR) has been reported[78]. Although EPCR has a structure highly homologous to that of CD1d[79], the TCR γδ



IPP



HMBPP

Fig. 1. Structure of isopenenylpyrophosphate, IPP (A) and (E)-4-hydroxy-3-methyl-but-2- enyl pyrophosphate (HMBPP) (B).

interacts with a region on the EPCR which is accessible only when the protein is partially unfolded. This finding raises the important question of what is the relevance of this recognition and which physiological conditions induce the expression of unfolded EPCR on the cell surface. Whether these reactivities are important to define the role of γδ cells is difficult to conclude, as diseases associated with γδ T cell deficiency have not been identified.

TCR γδ cells in infections

Human γδ T cells have been indicated as important effector cells in bacterial, viral and parasite infections. In many bacterial infections the number of circulating γδ T cells increases, such as in tuberculosis[80], tularemia[81], salmonellosis[82], and brucellosis[83]. The number of γδ T cells was also found increased in parasite infections including malaria[84], ehrlichosis[85], leishmaniasis[86], toxoplasmosis[87], and trypanosomiasis[88]. In these infections the expanded cells predominantly expressed the Vγ9Vδ2 TCR. Altered numbers of γδ T cells are frequently observed in some viral infections. In patients with AIDS, expansion of oligoclonal Vδ1-positive cells were described in the peripheral blood[89] and of Vδ2-positive cells in the lung[90]. It was proposed that in these patients γδ cells participate in the immune defense against opportunistic infections that are frequent in HIV-infected patients. Also in patients with cytomegalovirus infection, mostly after kidney transplant, the expansion of oligoclonal Vδ1- and Vδ3-expressing T cells has been observed. These γδ cells proliferate when stimulated with CMV-infected fibroblasts[91]. As some T cells expressing the Vδ1 chain recognize EPCR molecule[78], these cells could recognize these self-molecules on stressed cells as a mechanism of protection.

The function of γδ cells

γδ cells release large amounts of pro-inflammatory cytokines including IFN-γ, tumour necrosis factor (TNF)-α, chemokines such as Regulation on Activation, Normal T-cell Expressed and Secreted (RANTES) and macrophage inflammatory protein-1α (MIP1-α) when stimulated through TCR. The co-stimulation of toll like receptor (TLRs) expressed by γδ cells increased these effector functions[92]. The milieu where γδ cells are primed also contributes to the acquisition of different effector functions. The presence of IL-2 and lack of IL-4 induce differentiation of Th1-like γδ cells, whereas the presence of IL-4 and lack of IL-12 facilitate a Th2-like differentiation[93]. Recent studies emphasized the presence of γδ cell populations producing the pro-inflammatory cytokine IL-17[94], that participates in regulating neutrophil migration and inflammation. The release of this cytokine is largely independent of TCR activation and is facilitated by combined signaling of other cytokines, including IL-18 and IL-23. This mechanism of TCR-independent cell activation is shared with other innate-like cells, including iNKT cells and innate-like lymphoid cells, thus supporting the current knowledge that γδ cells behave as innate - like T cells. This conclusion is also supported by studies in which the freshly isolated Vγ9Vδ2 cells were first stimulated with phosphoantigens and then their gene expression profiles were studied using microarrays. Differential exposure to IL-2, IL-4 or IL-21 showed unique pleiotropy of γδ cells that transcribed differential cytokines and chemokines at the population level[95]. The expression of chemokine receptors has been investigated on different subset of γδ cells. Very few γδ cells express the lymph node homing receptor CCR7[96], whereas most of the cells express CXCR3 and CCR5. A small number of circulating γδ cells express number of CXCR1, CXCR2 and CX3CR1[97]. These patterns of chemokine receptor expression suggest that γδ cells present in blood preferentially migrate to the non-lymphoid tissue and at the sites of inflammation.

The available information suggests that γδ cells may attribute a number of unique functions that are different among other lymphocyte populations. First, their capacity to recognize microbial and self ligands is in agreement with a function of sentinel cells, which recognize metabolic alterations within other cells. This is supported by the activation of Vγ9Vδ2 cells interacting with other cells infected with bacteria and with altered mevalonate pathway[98]. The recognition of stressed cells that upregulate MICA and MICB proteins, two targets of Vδ1-expressing cells[99], together with their effector functions and the unique pattern of expressed chemokine and TLRs are in agreement with this hypothesis. A second important function of γδ cells is their capacity to fill the temporal gap between activation of innate and adaptive immune responses, when antigen-specific γδ cells are not activated and sufficiently expanded. A third function is contribution to maturation of professional APCs and of antigen-specific T cells into different effector subsets, as shown in a mouse model[100].

Mucosal associated invariant T (MAIT) cells

MAIT cells are innate-like cells and were first identified in the lymphocyte preparations from gut

lamina propria and lungs of mouse[101]. These T cells share some functional features with iNKT cells and in humans are abundant in blood (1-10% of circulating T cells), intestinal mucosa, mesenteric lymph nodes[102] and liver (25-40% of T cells)[103]. Like iNKT cells, MAIT cells express high levels of the NK-associated receptor CD161 and IL-18Rα[102,104], and ROR (γt) (ZBTB16 transcription factors[105]. However, unlike iNKT cells these are restricted by the monomorphic MHC class 1-related molecule (MR1)[101,106] and are characterized by a different development pathway[107].

MR1 structure and nature of bound antigens

The MR1 protein is highly conserved among mammals and its encoding gene is located within human chromosome 1, nearby the CD1 locus and, therefore, MR1 gene is not associated with the MHC[105]. MR1 is ubiquitously present in all cell types and its conservation suggests an important evolutionary conserved role in immunobiology[108,109]. Though MR1 shares sequence and structural similarity with the antigen-presenting molecules encoded by MHC-I and CD1 gene families, the structure of its antigen-binding groove is distinct from that of MHC-I and CD1 molecules[110]. The MR1 antigen-binding groove is made of charged and hydrophobic amino acid residues, with a clear deep pocket that has a small portal, probably used by bound antigens to protrude outside the pocket.

MAIT cells react to APCs infected with a variety of microbes, including several Gram-negative and Gram-positive bacteria, Mycobacteria, *Candida* and *Saccharomyces*[2,111]. Importantly, *Streptococcus* and *Listeria* species do not activate MAIT cells, indicating that the stimulatory antigen is conserved across many but not all microbial species. MAIT cells are also activated by fixed APCs co-cultured with *Escherichia coli*, suggesting that the antigen can be secreted by bacteria and may bind to MR1 on the surface of APC without internalization[2]. A recent study has shown that metabolic precursors of riboflavin, namely 6,7-dimethyl ribityllumazine, 6-methyl-7-OH-ribityllumazine, and 6-hydroxymethyl-ribityllumazine (Fig. 2) bind to MR1 and stimulate MAIT cells[110]. These metabolites are secreted by bacteria and a perfect correlation was found among the stimulatory bacterial species and their capacity to synthesize riboflavin. The size of these antigens is too small to fill the antigen-binding pocket of MR1, and, therefore, there is the possibility that other non-peptidic molecules may bind to MR1 and stimulate MR1-restricted T cells.

MAIT TCR repertoire and effector functions

MAIT cells express an evolutionary conserved semi-invariant TCRα chain (Vα7.2Jα33 in human and Vα19Jα33 in mice)[112,113], that is paired with a limited array of TCRβ chains, including Vβ2 and Vβ13 chains in humans and Vβ6 and Vβ8 chains in mice. This constrained TCR gene pairing, which is different from that of iNKT cells, suggests a unique mode of interaction with MR1-antigen complexes. Mutagenesis studies of MAIT TCRs with different Vβ and of the MR1 gene showed that a cluster of conserved amino acid residues in the TCR as well as on MR1 is crucial for MAIT cell activation[114].

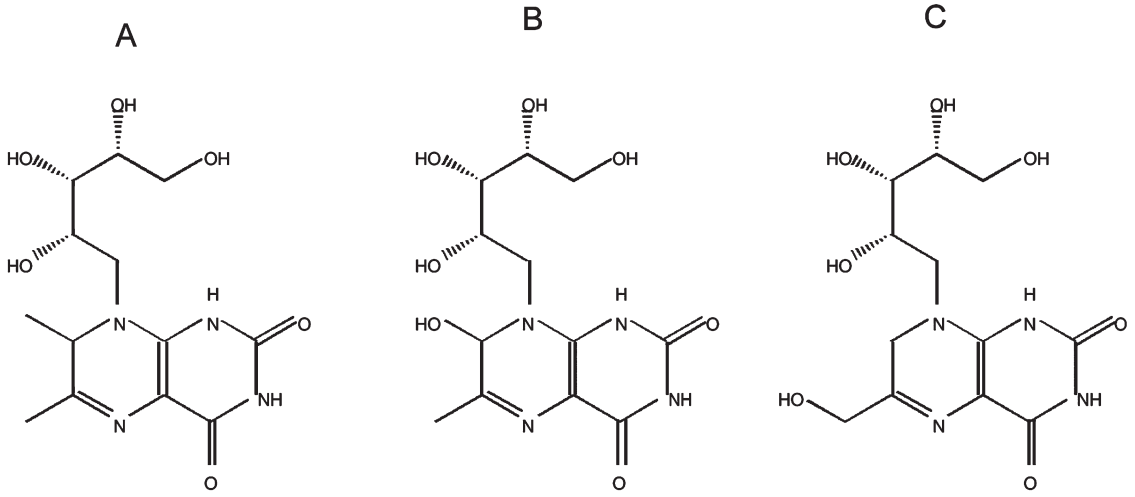


Fig. 2. Structure of 6,7-dimethyl-ribityllumazine **(A)**, 6-methyl-7-hydroxy-ribityllumazine **(B)**, and 6-hydroxymethyl-ribityllumazine **(C)**.

MAIT cells may show a phenotype of effector or memory cells. These cells produce cytokines such as IFN-γ, TNF-α, IL -4, IL-5, and IL-10 rapidly after their MR1-dependent activation[2,115,116]. Activated peripheral MAIT cells may also show a Th17-signature profile. These may release IL-17A and express the Th17-related chemokine receptor CCR6[104,105]. MAIT cells also express granzymes and Fas cell surface death receptor (FAS), suggesting a cytotoxic potential.

MAIT cells in infections

Human MAIT cells are naïve in cord blood and thymus, and expand after birth probably because of the colonization by commensal microbiota[102]. Germ-free mice do not have MAIT cells[101], highlighting the intimate relationship between MAIT cells and microbial flora. The fact that MAIT cells recognize microbial metabolites also suggests that these have an antimicrobial function[2,111]. It remains open whether these are activated during viral infections. MAIT cells protect mice infected with *Mycobacterium abscessus* or *E. coli*[2] and MR1-deficient mice have reduced capacity to clear *Klebsiella* as compared to control animals[117]. In humans infected with *M. tuberculosis,* considerably less MAIT cells are found in the peripheral blood[2,111], whereas these cells accumulate in the lung at the site of infection[111]. Recently, two independent studies have shown that MAIT cell numbers are highly decreased in the blood of HIV patients and their numbers do not recover after anti-retroviral therapy[118,119]. Whether these changes reflect the accumulation of MAIT cells in tissues or their exhaustion and disappearance remains unknown. In multiple sclerosis patients, MAIT cells were found increased in one study[120] and decreased in a second one[121]. It remains unclear whether these changes are associated with differences in the genetic background, diet or gut microbiome.

Modifications of MAIT cell numbers in other clinical conditions such as diabetes, lupus erythematosus and inflammatory bowel disease have also been suggested[107,122,123]. Although it is unclear whether MAIT cells are involved in the pathogenesis of these diseases, yet the changes in their numbers are strongly indicative of their participation in the immune response in these diseases. All together, these studies suggest that MAIT cells play a major role at the interface of host-microbe interactions and contribute to immune system homeostasis.

Conclusion

In humans a large fraction of circulating and tissue resident T cells recognize non-peptidic antigens. In most of the cases non-peptidic antigens are of microbial origin, suggesting a co-evolution of these antigen specificities with pathogenic and commensal microbes. The strategies to recognize non-peptidic antigens are diverse and utilize at least three different families of antigen-presenting molecules. This multiplicity of presentation mechanisms is an indication of the flexibility evolved by the immune system to focus the TCR on different antigenic molecules. The disclosure of the molecular interactions between TCR and complexes formed by the presenting molecules and non-peptidic antigens may reveal novel features of TCR activation and may provide useful insights on how these immune responses are regulated. Another important feature is the relatively conserved nature and essential metabolic function of non-peptidic antigens. The important physiological functions of these metabolites secure the immune response from the generation of microbial mutants lacking these metabolites and thus escaping the immune response. Finally, the identification of non-peptidic antigens may offer new opportunities in the fields of immunotherapy and vaccination.

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