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# The role of donor-unrestricted T-cells, innate lymphoid cells, and NK cells in anti-mycobacterial immunity

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## Abstract

Vaccination strategies against mycobacteria, focusing mostly on classical T- and B-cells, have shown limited success, encouraging the addition of alternative targets. Classically restricted T-cells recognize antigens presented via highly polymorphic HLA class Ia and class II molecules, while donor-unrestricted T-cells (DURTs), with few exceptions, recognize ligands via genetically conserved antigen presentation molecules. Consequently, DURTs can respond to the same ligands across diverse human populations. DURTs can be activated either through cognate TCR ligation or via bystander cytokine signaling. TCR-driven antigen-specific activation of DURTs occurs upon antigen presentation via non-polymorphic molecules such as HLA-E, CD1, MR1, and butyrophilin, leading to the activation of HLA-E–restricted T-cells, CD1-restricted T-cells, mucosal-associated invariant T-cells (MAITs), and TCRγδ T-cells, respectively. NK cells and innate lymphoid cells (ILCs), which lack rearranged TCRs, are activated through other receptor-triggering pathways, or can be engaged through bystander cytokines, produced, for example, by activated antigen-specific T-cells or phagocytes. NK cells can also develop trained immune memory and thus could represent cells of interest to mobilize by novel vaccines. In this review, we summarize the latest findings regarding the contributions of DURTs, NK cells, and ILCs in anti–*M tuberculosis*, *M leprae*, and non-tuberculous mycobacterial immunity and explore possible ways in which they could be harnessed through vaccines and immunotherapies to improve protection against Mtb.

**KEYWORDS**
donor-unrestricted T-cells, HLA-E, innate lymphoid cells, *Mycobacterium tuberculosis*, NK cells, vaccine

## 1 | HLA-E–RESTRICTED T-CELLS

The non-classical HLA class Ib molecule HLA-E is best known as a ligand for CD94/NKG2A and CD94/NKG2C, which are inhibitory and activating C-type lectin receptors, respectively, that are expressed by NK cells and some subsets of T-cells.[1] CD94/NKG2A recognition of HLA-E–presented self-peptides, typically derived from HLA class Ia signal sequences, leads to inhibition of NK-cell

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cytolytic function.[2,3] This interaction operates as a surveillance system for monitoring bonafide surface expression of HLA class Ia, which can be downregulated by tumors or pathogens as an immune escape mechanism.[4] However, HLA-E molecules have also been shown to present peptides of non–self-origin, such as the glycoprotein UL40 leader peptide from CMV, which, being identical to HLA class Ia signal peptide, also engages CD94/NKG2A allowing CMV to escape NK-cell–mediated cytotoxicity.[5] Moreover, HLA-E–dependent peptide presentation to TCRs on human $CD8^+$ T-cells has been shown for a variety of bacterial and viral pathogens including Mtb, Salmonella, and CMV.[6-12] Mtb-specific HLA-E–restricted T-cells were able to control intracellular mycobacterial growth in human macrophages ex vivo, rendering these cells interesting targets for vaccination strategies.[9]

In contrast to other HLA molecules, HLA-E is essentially monomorphic: only one amino acid located outside of the peptide-binding groove differentiates the two allelic, coding variants in humans, HLA-E*01:01 (107Arg) and HLA-E*01:03 (107Gly).[13] These two allelic variants are equally represented in human populations, promoting interest in HLA-E as a vaccine presentation molecule conserved in otherwise genetically diverse populations.[14] HLA-E–mediated antigen presentation has attracted particular interest in the context of Mtb infection after several studies showed that HLA-E, in contrast to classical HLA molecules, was resistant to HIV-mediated downregulation.[15] Although a recent publication showed that this resistance varied with the HIV strain studied,[16] the observed maintained HLA-E expression can be of significance when administering vaccines to individuals at risk of Mtb/HIV coinfection. HLA-E's function can be studied in mice and non-human primates, due to the high structural and functional conservation across vertebrates, with Qa-$1^b$, Mamu-E, and Mafa-E being the HLA-E orthologues in mice, rhesus macaques (RM), and cynomolgus macaques, respectively.[17,18] This is particularly relevant for preclinical and translational HLA-E–oriented vaccine research.

### 1.1 | Functional properties of HLA-E–restricted T-cells in an infectious disease: the example of Mtb infection

HLA-E tetramers loaded with Mtb-derived peptides were used to detect circulating Mtb-specific HLA-E–restricted T-cells in active TB patients, particularly in TB patients coinfected with HIV, but at lower frequencies also in LTBI. The higher frequencies of these cells in active TB and HIV/TB patients suggest activation during disease, possibly correlating with disease severity.[9,19] Functional characterization of these cells ex vivo and of Mtb-specific HLA-E–restricted polyclonal and monoclonal T-cells in vitro revealed an unorthodox Th2 cytokine and effector-memory profile, with both cytotoxic and regulatory activities. Despite possessing Th2-like characteristics, these T-cells were able to inhibit intracellular mycobacterial growth in human Mtb-infected macrophages.[8,9] In mice, balanced activation of Qa-$1^b$–restricted T-cells was found to be associated with improved control of bacterial burden, reduced inflammation, and reduced mortality following Mtb infection.[20] In line with these cells' Th2 characteristics, our own work further demonstrated that Mtb-specific human HLA-E–restricted T-cells could activate B-cells,[9] which play a key role in T-cell immunity to Mtb.[21] Mtb-specific HLA-E–restricted T-cells, however, also can express IFN-γ, a Th1 cytokine, which has been associated with control of Mtb growth.[7,11,22] Taken together, the polyfunctional nature of Mtb-specific HLA-E–restricted T-cells renders them interesting targets for vaccine-inducible control of Mtb infection, by mobilizing a number of as yet incompletely resolved functional pathways, which include helping macrophages, promoting B-cell activation, and regulating excessive inflammatory responses (Table 1).

### 1.2 | Studies on HLA-E–restricted T-cells in experimental vaccination studies

The potential of HLA-E–restricted T-cells in vaccination strategies is further supported by a series of studies in RM demonstrating the induction of these unconventional T-cells after vaccination with engineered rhesus cytomegalovirus (RhCMV) vectors. These vectors carried virus or parasite antigens and elicited tissue-resident and high-frequency circulating effector-memory $CD4^+$ and $CD8^+$T-cell responses specific for the pathogen-derived antigen.[23] Hansen et al[24] showed that vaccination of RM with RhCMV/simian immunodeficiency virus (SIV) led to complete protection against high-dose SIV challenge in half of the animals, which was at least partially dependent on Mamu-E–restricted T-cell responses. Mamu-E–restricted T-cells from these animals were able to recognize SIV-infected $CD4^+$T-cells as shown by IFN-γ and TNF-α production, a response which could be blocked by the HLA-E–specific binding VL9 peptide. Similarly, vaccination of RM with RhCMV/hepatitis B virus (HBV) elicited Mamu-E–restricted T-cells producing IFN-γ upon recognition of both RM- and human-derived HBV-infected primary hepatocytes in vitro.[24,25] *Plasmodium knowlesi* (Pk) challenge of RhCMV/Pk-vaccinated RM led to delayed parasitemia, again partially dependent on Mamu-E–restricted T-cells.[26] A significant reduction in the magnitude of Mtb infection and disease after RhCMV/TB vaccination in RM was observed compared with unvaccinated animals.[27] In this case, Mamu-E- and MHC-II- or MHC-Ia–restricted Mtb-specific effector T-cell responses were induced, partly depending on the vector used, and both may play a role in protection, hinting to diverse and possibly complementary protective mechanisms in the immune response against Mtb.[27] The finding of MHC-E–restricted SIV epitopes that were recognized by all tested (outbred) animals led to the term "supertopes" and supports the potential of HLA-E–targeting vaccines to induce effector T-cell responses regardless of human genetic diversity.[24]

Recent insights into HLA-E peptide-binding motifs[28,29] and the availability of improved peptide/HLA-E–binding algorithms will allow for better selection of optimal peptide ligands for HLA-E from diverse pathogens and perhaps even tumors, which could be

**TABLE 1** Activation mechanisms and functional relevance of DURTs, NK cells, and ILCs in the context of mycobacterial infections

| | | | | CD1-restricted T cells | | | ILCs | |
|---|---|---|---|---|---|---|---|---|
| | **HLA-E–restricted T-cells** | **MAIT cells** | **TCRγδ T-cells** | **Group 1 CD1-restricted T-cells** | **(i)NKT cells** | **NK cells** | **ILC1s** | **ILC3s** |
| Activation via antigen | Peptides: | Metabolic derivatives: | Phosphoantigens: | Lipids: | | UL-16–binding protein (ULBP)1 | | |
| | self and pathogen-derived | 5-OP-RU | IPP, HMBPP, mGLP | GMM, MA, SGLs, GroMM | α-GalCer, PIM | | | |
| Activation via cytokines (TCR-independent) | | IL-12/IL-18 | | | IL-12/IL-18 | IL-12/IL-18 | IL-12/IL-15/IL-18 | IL-23 |
| Functional relevance | Mtb growth inhibition | Recruitment to site of infection | Mtb growth inhibition | Mtb growth inhibition | Mtb growth inhibition | Mtb growth inhibition | IFN-γ production | Th17 profile |
| | Cytotoxic, regulatory, and helper functions | Regulatory function | Cytotoxic function (perforin and granulysin) | Cytotoxic function (perforin, granzyme B, and granulysin) | Th1 and Th17 cytokine profiles | Trained immunity: IL-1β, IL-6, and TNF-α production | | |
| | Th1 and Th2 cytokine profiled | Cytotoxic function (granzyme B) | IFN-γ, GM-CSF, IL-3, TNF-α production | Th1-like cytokine profile | | Regulatory function | | |
| | | | Immunoregulatory role (IL-10) | CD40L, IFN-γ, IL-2, TNF-α, and IL-17a production | | Cytotoxic function | | |
| | | | Th17 cytokine production | | | Memory-like function | | |
| | | | Helper function | | | IFN-γ production | | |

harnessed in better vaccines and therapeutics. Further research will be necessary to define the influence of peptide-binding affinity and stability on optimal TCR triggering via HLA-E. An additional advantage when considering HLA-E as a vaccine target, especially in the case of intracellular pathogens such as Mtb, is HLA-E's expression in the Mtb phagosome of macrophages.[30] Therefore, the Mtb phagosome could be involved in HLA-E peptide loading and thus be an alternative cytosolic cross-presentation pathway, although the precise mechanisms involved remain undefined.

## 2 | MUCOSAL-ASSOCIATED INVARIANT T (MAIT) OR MR1-RESTRICTED T-CELLS

MAIT-cells are a subset of (mostly oligoclonal) TCRαβ T-cells initially associated with mucosal surfaces, and defined by surface expression of CD161, CD26, and a semi-invariant TCR.[31,32] MAIT-cells express chemokine receptors CCR9, CCR5, CCR6, and CXCR6, suggesting preferential homing to liver, lung, and intestines.[33] However, MAIT-cells also circulate in the blood from where they are recruited during bacterial infections such by Mtb.[34] MAIT-cells have the capacity to detect bacterially infected cells in mice and humans through the recognition of metabolic intermediates presented by the monomorphic MHC-related protein 1 (MR1). Known MAIT agonists include riboflavin metabolites, which are only present in bacteria and fungi, such as 5-OE-RU and 5-OP-RU,[35] making them an important component of the mucosal immune response against bacterial infections.

Despite the initially observed conservation of the MAIT TCR repertoire, additional studies using MR1 tetramers discovered more heterogeneous intra- and inter-individual TCR-V fragment usage.[36-38] Comparison of Mtb-specific MR1 tetramer–positive and MR1 tetramer–negative MAIT-cells showed that MAIT-cells can react to diverse ligands.[39] Nevertheless, the relatively limited MAIT TCR repertoire and its conservation across species are clear indications of underlying conserved specificity and functionality of at least a subset of MAIT-cells. A recently expanded MAIT-cell classification has suggested to encompass the apparent increasing diversity of human MAIT-cell populations, considering MR1 restriction, antigen reactivity, and innate-like effector function associated with the expression of promyelocytic leukemia zinc finger (*PLZF*).[31] Classical MAIT-cells can be identified by the expression of a semi-invariant TCR encoded by *TRAV1-2* and TRAJ33/12/20 α-chain, with restricted TCRβ chain usage dominated by *TRBV6* and *TRBV20*. The alternative, more variable expression of *TCRA* genes in TRAV1-2–negative MAIT-cells characterizes non-classical MAIT-cells. Atypical MR1-restricted T-cells can express variable TCR fragments and are phenotypically distinct from MAIT-cells as determined by reactivity to alternative antigens and lack of PLZF expression.[31] Therefore, MAIT-cells are also often referred to as MR1-restricted T-cells (MR1T), but it is currently uncertain whether all MR1-restricted T-cells classify as MAIT-cells.

Classical MAIT-cells share high similarity in transcriptional patterns with invariant natural killer T (iNKT)-cells, which we discuss in detail below, and both types of unconventional T-cells express a transcriptome distinct from that of conventional $CD8^+$T-cells.[39,40] These shared transcriptional programs are acquired during thymic development and reflect defined effector functions and tissue residency.[41] Upon mycobacterial activation, MAIT-cell gene expression profiles overlapped also with that of activated NK cells and conventional $CD8^+$T-cells, further suggesting MAIT-cells as a functional bridge between innate immunity and adaptive immunity.[42]

### 2.1 | Contribution of MAIT-cells to protective immunity against mycobacterial infections

Circulating MAIT-cells were decreased in active TB patients compared with healthy controls, a trend also observed in HIV- and HIV/TB-infected patients. In contrast, MAIT-cells were enriched at the site of infection, suggesting preferential homing from the blood.[43-48] In a recent report, MAIT-cells were enriched in bronchoalveolar lavage (BAL) fluids of active TB patients compared with uninfected individuals and were reactive to *Mycobacterium smegmatis*–infected antigen-presenting cells only when MR1 was expressed.[49] However, a recent study conducted in a high endemic setting detected no differences in circulating MAIT-cell frequencies when comparing TB patients with LTBI and healthy Mtb–exposed controls.[50] These differential frequencies of MAIT-cells across different studies suggest a potential effect of (high) Mtb exposure or other environmental factors such as microbiota, nutritional status, co-infections, or host genetics. In any case, these studies suggest that MAIT-cells may not be an appropriate correlate of Mtb infection in high-transmission areas. Moreover, increased susceptibility to meningeal TB in a Vietnamese cohort was associated with a single genetic polymorphism causing increased MR1 expression, while reduced MR1 expression of MR1 was associated with increased mortality. This suggests a role for MR1-mediated antigen presentation and MAIT-cell activation in human TB susceptibility, and perhaps also dissemination to the central nervous system.[51]

Functionally, MAIT-cells can be cytotoxic toward bacterially infected MR1-expressing lung-epithelial cells.[43,52] In recently Mtb-exposed subjects resistant to infection, MAIT-cells showed an activated phenotype with expression of granzyme B and IL-2RA.[53] The reduced frequency of MAIT-cells in the blood of active TB patients was linked to the elevated expression of exhaustion marker programmed cell death protein 1 (PD-1) on MAIT-cells[54] and to deficient MAIT-cell production of IFN-γ, TNF-α, granulysin, granzyme B, and IL-17F when exposed to antigen, compared with healthy controls.[55,56] In contrast, a recent study showed that IFN-γ production by circulating MAIT-cells in response to Mtb was higher in active TB patients and LTBI compared with uninfected contacts, although LTBI showed highest IFN-γ production, suggesting a possible role for MAIT-cells in infection control.[57] In another study, MAIT-cells isolated from tuberculous pleural effusions displayed increased cytokine and cytotoxic responses to Mtb-derived antigens compared with blood MAIT-cells, mainly mediated by IL-2, IL-12, and IL-18

signaling, supporting the importance of Mtb-specific and bystander activation of MAIT-cells at the site of infection.[58] Interestingly, the persistent absence of IFN-γ release assay (IGRA) conversion in a sizeable fraction of highly Mtb-exposed household contacts was associated with a reduced proportion of IL-17–producing circulating MAIT-cells compared with IGRA converters, an indication of the possible importance of migration of these cells to the infection site for bacterial control, although this could not be proven.[59] In children, where TB disease is usually the result of primary infection as opposed to the reactivation of latent infection predominantly seen in adults, active TB correlated with reduced MAIT-cell numbers in blood and BAL fluid compared with LTBI and healthy children.[60] Recently, activated, proliferating, granzyme B–producing MAIT-cells were identified in the blood of NHP after BCG vaccination and Mtb infection, in agreement with observations in humans.[61] In another study, a transient increase in MAIT-cells was detected in BAL but not peripheral blood after protective intravenous BCG vaccination in NHP, suggesting that MAIT-cell recruitment to the infection site might be involved in protection against aerosol Mtb challenge.[62] Exploration of lung tissue and TB granulomas of these Mtb-infected macaques showed an uneven accumulation of activated MAIT-cells. Kauffman *et al* found that MAIT-cells were increased in the lungs of only half of the NHP at late time points of Mtb infection. Moreover, in TB granulomas only few and poorly activated MAIT-cells were identified, which expressed low levels of granzyme B, suggesting a limited role for MAIT-cells in Mtb control.[63] Bucsan *et al*, on the other hand, found increased MAIT-cell frequencies with Th1 cytokine profiles in blood and BAL fluid of NHP only at early time points after Mtb infection.[64]

Altogether, these studies underscore the pertinence of MAIT-cells for mucosal immunity and the ambiguity of their role in local immune control of Mtb infection and TB disease progression in humans. Importantly, we must consider the spectrum, and the kinetics of Mtb infection and TB disease development when designing strategies to mobilize MAIT-cells, as they might have an important short term and early role at the site of Mtb infection, with possibly reduced functionality in more advanced TB disease.

## 2.2 | MAIT-cell activation pathways and potential as vaccine or immunotherapeutic target

Studies in mice have shed further light on the function of MAIT-cells during infection. MR1-deficient mice showed increased susceptibility to *Mycobacterium abscessus* and *Escherichia coli* infections.[44] Follow-up studies demonstrated a protective function of mouse MAIT-cells against infections with *Klebsiella pneumoniae*, *Mycobacterium bovis BCG*, *Francisella tularensis*, and *Legionella longbeachae*.[65-68] In vitro activation of human MAIT-cells primarily depended on MR1 antigen presentation and subsequent TCR signaling at early time points, while combined IL-12/IL-18 signaling was shown to be equally important at later time points, supporting a TCR-independent mechanism of later-phase MAIT-cell stimulation.[69] This bystander activation mechanism was also observed in BCG-specific MAIT-cells, hinting to a role for these cells in innate immunity cytokine-driven expansion in the response against Mtb infection.[70]

The impaired control of bacterial loads in genetically MR1-deficient mice compared with wildtype mice at early time points after *Francisella tularensis* infection provided strong evidence for a role of MAIT-cells in early innate immunity and in initiating adaptive immune responses to chronic infection through IFN-γ production and the recruitment of $CD4^+$ and $CD8^+$T-cells.[67] The importance of MAIT-cells in protection against bacterial infections was further substantiated by experiments in which MAIT-cells were adoptively transferred into immunodeficient mice, leading to rescue from lethal *Legionella* infection.[68] Importantly, in vivo MAIT-cell priming with 5-OP-RU and TLR9/2 agonists CpG or $Pam_2Lys$ increased protection against *Legionella* infection.[68] In contrast, 5-OP-RU–mediated expansion of MAIT-cells prior to Mtb infection in mice was associated with delayed Mtb-specific $CD4^+$T-cell priming and lack of protection, suggesting that MAIT-cell priming might have pathogen-specific effects.[71] During Mtb infection in vivo, the presence of inhibitory MR1-ligands such as riboflavin and FO might counteract the effect of MAIT-cell agonists,[72] resulting in a balanced MAIT-cell response, which, however, might limit protection against Mtb despite successful vaccine-mediated priming. In contrast, IL-17A–mediated control of Mtb in vivo was achieved through administration of MAIT agonist during chronic infection, pointing to differential functions for MAIT-cells during steady and inflammatory states.[71] In two recent studies, accumulation of MAIT-cells in BCG- and Mtb-infected mouse lungs was shown to be dependent on exposure to the MAIT-cell–activating antigen 5-OP-RU and TLR2/6 agonist $Pam_2CSK_4$ or $Pam_2Cys$.[73,74] However, this was associated with a reduced ability to control Mtb, and with increased levels of anti-inflammatory cytokine IL-10, adding to the complexity of targeting MAIT-cells to enhance host protection against Mtb infection.[73,74] In the context of Mtb/SIV coinfection in NHP, MAIT-cells were recruited to granulomas, but were functionally impaired in vitro and in vivo.[75] Impaired MAIT-cell responses to Mtb were also observed in HIV-infected patients and could be redressed through IL-10 signaling blockade.[76] Therefore, a proper balance between induction and regulation of MAIT-cells linked to the inflammatory environment during MAIT-cell recruitment seems to be crucial for an optimal response during Mtb infection.

Additionally, host gut microbiota has been shown to contribute to MAIT-cell function in the early protection of Mtb lung colonization in mice and humans.[53,77] Indeed, the development of MAIT-cells that are relevant for tissue repair depended on the exposure to riboflavin-derived antigens present in early-life microbiota.[78] This is further supported by the finding that MAIT-cell frequencies increased rapidly after birth and with age, regardless of BCG vaccination status, hinting to a role of gut microbiome colonization and increased microbial exposure in MAIT-cell expansion.[79]

In conclusion, although MAIT-cells have the capacity to eliminate bacteria-infected cells in vitro, their specific role in overall protection against Mtb in humans and NHP remains unresolved. Notably, MAIT-cells might be particularly important for mucosal

immunity at early time points after primary Mtb infection, by their mobilized effector functions or by regulating other adaptive immune cells (Table 1). Additional larger longitudinal studies will be necessary to unravel MAIT-cells' roles, specific effector functions, and homing behavior in host defense against mycobacterial infection, and to identify effective MAIT-cell–targeting strategies to increase protection at different stages of Mtb infection or TB disease. Moreover, it will be necessary to evaluate MR1 ligands as vaccine components, in particular on the feasibility and potential for MAIT-cell expansion upon vaccination. Presently, the possibility to target MAIT-cell responses with other than whole-cell vaccines hinges upon the development of stable MR1 ligands. Furthermore, enhancing MAIT-cell priming with cytokine costimulation or blockade, perhaps immune-checkpoint manipulation, might represent alternatives to improve protection against Mtb, although this remains to be evaluated.

## 3 | TCRγδ T-CELLS

TCRγδ T-cells are $CD3^+$T-cells expressing Vγ and Vδ-encoded TCR, and are unique in their capacity to recognize non-peptidic phosphorylated intermediates of metabolic pathways, known as phosphoantigens, presented via butyrophilin (BTN).[80] BTN-mediated presentation requires binding of phosphoantigen to the intracellular domain of BTN3, known as B30.2, inducing conformational changes in the BTN2/3 extracellular domains recognized by TCRγδ T-cells.[81,82] TCRγδ T-cells react to pathogen-derived phosphoantigens such as isopentenyl pyrophosphate (IPP),[83] (E)-4-hydroxy-3-methyl-but-2-enyl pyrophosphate (HMBPP),[84] and 6-O-methylglucose lipopolysaccharides (mGLP), the latter being able to promote Mtb inhibitory TCRγδ T-cell activity in vitro.[85] Moreover, a small population of TCRγδ T-cells reactive to CD1d-presented α-GalCer has been described[86]; also, Mtb-derived protein antigens recognized by TCRγδ T-cells have been identified, further broadening the possibilities to activate TCRγδ T-cell in the context of Mtb infection.[87,88]

TCRγδ T-cells were more abundant in peripheral blood of TB patients compared with healthy controls and to patients with other lung infections or malignancies.[89] Repertoire sequencing allowed the detection of clonal expansion of TCRγδ T-cells in blood and lungs of TB patients.[87,90] Reactivity of TCRγδ T-cells to Mtb in vitro was higher in individuals with less severe TB and was associated with the production of anti-microbial IFN-γ, GM-CSF, IL-3, and TNF-α.[91] In a different study, TB severity was associated with $Vγ9Vδ2^+$ T-cell production of IL-10, suggesting an immunoregulatory role for these cells, which could be unfavorable for protection.[92] A predominant subset of $Vγ9Vδ2^+$ TCRγδ-expressing cells representing up to 5% of circulating T-cells in healthy individuals can expand in patients with bacterial and parasitic infections and represent up to 20%-50%, highlighting their participation in immune responses against infections.[93] Supporting a possibly protective role for TCRγδ T-cells during Mtb infection, IFN-γ–producing circulating $Vγ9Vδ2^+$ effector T-cells were reduced in acute pulmonary TB patients compared with healthy donors,[94] and decreased levels of circulating $Vδ2^+$ TCRγδ T-cells were correlated with more severe pulmonary lesions in TB patients.[92,95] Similarly, hyporesponsive $Vγ9Vδ2^+$ were identified in TB patients' lungs.[96] A more recent study showed an association between the establishment of latent Mtb infection in exposed household contacts and a defective activation of TCRγδ T-cells, characterized by impaired upregulation of PD-1 and CD69, an effect which was not observed in persistently IGRA-negative household contacts.[53]

In NHP, where recognition of phosphoantigens by TCRγδ T-cells is conserved,[97] protection against Mtb challenge was associated with a rapid and transient expansion of BAL $Vγ9^+$T-cells after intravenous BCG vaccination.[62] The expansion of $Vγ2Vδ2^+$ TCRγδ T-cells associated with Mtb clearance and increased survival after challenge of BCG-vaccinated macaques was linked to Th17 cytokines.[98] In TB patients, frequencies of circulating IL-17–producing $Vγ9Vδ2^+$ T-cells were increased compared with controls.[99] Upon BCG vaccination, an increased number of circulating IFN-γ–producing TCRγδ T-cells were detected in newborns and infants.[100,101] Importantly, TCRγδ T-cell responses to secondary stimulation were enhanced in adults after BCG vaccination compared with unvaccinated controls.[102] These studies suggest a memory TCRγδ T-cell function supporting the potential of TCRγδ T-cells as vaccine targets.

Mechanistically, the coculture of $Vγ9Vδ2^+$ T-cells with Mtb- or BCG-infected macrophages led to control of intracellular mycobacterial growth and to killing of infected macrophages through perforin and granulysin production in vitro,[103,104] or by inducing monocyte TNF-α production through granzyme A.[105] Moreover, Mtb phosphoantigen–activated $Vγ9Vδ2^+$ T-cells could induce DC maturation through TNF-α and IFN-γ production.[106] The early IFN-γ production by TCRγδ T-cells in mice lungs was associated with optimal DC activation in vivo.[107] The helper function of Mtb-specific $Vγ9Vδ2^+$ T-cell lines was assessed in vitro, showing that CD40-CD40L interactions were necessary for $Vγ9Vδ2^+$ T-cells to promote TCRαβ T-cell–mediated control of Mtb infection.[108] A regulatory function for TCRγδ T-cells has also been suggested, as IL-22–producing T-cells associated with severe TB were downmodulated by phosphoantigen-stimulated TCRγδ T-cells, through an IFN-γ–mediated mechanism.[109] Thus, in attempts to identify the mechanisms involved in TCRγδ T-cell–mediated protection against Mtb, these collective studies suggest that cytotoxic, regulatory, and helper effector functions of TCRγδ may all be involved (Table 1).

Several studies have explored a number of different strategies to target TCRγδ T-cell responses to improve protection against Mtb before and after infection. NHP vaccinated with ESAT6-Ag85B fusion protein combined with phosphoantigens induced a fast Th1-like TCRγδ T-cell expansion upon immunization.[110] Treatment of NHP with HMBPP phosphoantigen in combination with IL-2 led to a long-lasting $Vγ9Vδ2$ effector-memory T-cells expansion in the lungs, with the capacity to produce IFN-γ and perforin upon restimulation.[111] In another study, $Vγ9Vδ2^+$ T-cells induced by an attenuated HMBPP-producing *L monocytogenes*

contributed to reduced pulmonary and systemic bacterial burden in Mtb-challenged NHP. This vaccine elicited Vγ9Vδ2$^+$ T-cells capable of inhibiting intracellular Mtb growth through IFN-γ and perforin production.[112,113] Postinfection, TCRγδ-centered therapy has also been explored through the adoptive transfer of Vγ9Vδ2$^+$ T-cells after Mtb infection in NHP, inducing decreased pulmonary and systemic bacterial burden and attenuated TB morbidity.[114] The possibility of modulating TCRγδ T-cell responses with cytokines has additionally been explored in different studies. For instance, IL-15 enhanced the proliferation of TCRγδ T-cells and together with IL-12 induced IFN-γ production in vitro.[115] IL-12–mediated expansion of Vγ9Vδ2 T-cells in vitro led to IFN-γ or TNF-α–dependent control of intracellular mycobacteria growth.[116] In NHP, phosphoantigen administration in combination with IL-2 during early pulmonary Mtb infection induced protective multifunctional IFN-γ, granulysin, and perforin-producing Vγ9Vδ2$^+$ T-cells in blood and lungs, with IL-12 production by these cells possibly further enhancing conventional T-cell responses.[117] Therefore, diverse strategies have shown promise to target protective TCRγδ T-cell responses against Mtb, both pre-infection and postinfection.

## 4 | CD1-RESTRICTED T-CELLS

T-cell recognition of the molecularly highly complex and uniquely structured Mtb envelope is possible through presentation of lipid-derived antigens via the CD1 family of monomorphic antigen-presenting molecules. Lipids are essential components of mycobacterial cell envelopes and can therefore represent unique targets for DURT-mediated immunity against Mtb.

The CD1 family includes CD1a, CD1b, and CD1c (group 1), and CD1d (group 2)[118,119] molecules, complemented by the intracellular lipid transfer protein CD1e (group 3), which is not directly involved in antigen presentation.[120] Group 1 and group 2 CD1 molecules can present antigen to CD4$^+$ and CD8$^+$T-cells (as well as in some cases double-negative T-cells), and to both TCRαβ- and TCRγδ-expressing T-cells. CD1 molecules recycle in the lysosomes where they encounter phagocytosed bacteria, allowing them to bind lipids and return to the cell surface for presentation.[121] CD1a recycles via early endosomes,[122] while cytoplasmic tyrosine motifs localize CD1b to late endosomes and lysosomes for lipid loading.[123] CD1c and CD1d are instead regulated by adapter protein 2 (AP-2) to survey different intracellular compartments, and localize to early and late endosomes.[124] Group 2 or CD1d-restricted T-cells are commonly known as natural killer T (NKT) cells and can be further classified on antigen specificity and on TCR diversity.

Despite their high structural similarity, the presentation of lipid antigens via group 1 and group 2 CD1 molecules activates functionally distinct CD1-restricted T-cells (Table 1). The presence of lipid-specific polycytotoxic T-cells in peripheral blood and BAL was associated with Mtb infection control.[125] The expression of CD1b and CD1d on DCs and macrophages in human TB granulomas supports a role for lipid-specific T-cell responses during TB and hints to the possibility of modulating their response to enhance immunity.[126,127]

### 4.1 | Function of group 1 CD1-restricted T-cells and potential as vaccine targets

CD1a-restricted T-cells can recognize Mtb-derived lipopeptide dideoxymycobactin (DDM).[128,129] CD1b-restricted T-cell responses have been defined mostly in the context of mycolic acid (MA),[130] glucose monomycolate (GMM),[121] sulfoglycolipids (SGLs, Mtb-specific),[131] and glycerol monomycolate (GroMM)[132] recognition. GMM-reactive T-cells have been specifically associated with active mycobacterial infection, as its synthesis requires mycobacterial mycolate and host glucose.[133] CD1c-restricted T-cells can respond to isoprenoid glycolipids and mycoketide antigens.[134,135] The development of lipid-loaded tetramers for studies focused around group 1 CD1-restricted T-cells facilitated the demonstration of comparable frequencies of lipid-reactive, CD1a-, CD1b-, and CD1c–restricted T-cells in TB patients or LTBI.[136-138]

Several studies have explored the role of group 1 CD1-restricted T-cells in immunity against Mtb. Initial work demonstrated Mtb-specific Th1-like cytokines and granulysin-mediated cytotoxicity by CD1-restricted T-cell clones in vitro.[119,139] CD1b-restricted CD8$^+$T-cells from Mtb-infected patients, but not uninfected controls, produced IFN-γ upon SGL stimulation leading to intracellular Mtb killing.[131] Expansion of CD1-restricted T-cells was associated with bacterial burden, as MA-stimulated circulating and BAL T-cells from TB patients but not BCG-vaccinated healthy controls produced IFN-γ and IL-2, a response which declined following completion of treatment.[140] Similarly, activated GMM-specific CD1b-restricted T-cells produced proinflammatory cytokines IFN-γ and TNF, supporting their anti-microbial effector function.[141] On the other hand, CD1b-restricted GroMM-specific CD4$^+$T-cells were detected in BCG-vaccinated and LTBI but not active TB patients, suggesting that these cells might be functionally relevant during Mtb exposure and disappear from the circulation or become anergic or exhausted during active TB.[132] Similarly, CD1b-restricted T-cells were associated with Mtb exposure rather than TB disease as higher frequencies of MA- and GMM-loaded CD1b tetramer$^+$ cells were identified in highly Mtb-exposed individuals compared with low or unexposed controls, while no differences were observed between TB patients, LTBI, and uninfected individuals.[142] Ex vivo profiling of GMM-specific T-cells in healthy and Mtb-infected individuals showed a polyfunctional CD4$^+$T-cell phenotype expressing CD40L, IFN-γ, IL-2, TNF-α, and IL-17a.[143] Moreover, lipid-reactive polycytotoxic CD8$^+$T-cells capable of controlling intracellular Mtb growth by expressing perforin, granzyme B, and granulysin were found in BAL-cells from LTBI.[125] The relevance of CD1-mediated immunity to Mtb is further supported by population genetic studies in Vietnam in which increased TB susceptibility was associated with an intronic *CD1A* polymorphism linked to functional deficiency.[144] GMM-specific T-cells from LTBI

were shown to persist and express the memory marker CD45RO,[145] and MA-specific T-cells could be expanded 1-2 years after curative treatment, indicating memory function[140] and supporting the potential for targeting these cells by vaccination. Altogether, these studies show that lipid-reactive CD1-restricted T-cells are an important component of the cellular immune response to Mtb.

Group 1 CD1 molecules are naturally expressed in guinea pigs, a TB model in which vaccination with mycobacterial lipids led to a reduction in Mtb burden and lung pathology after aerosol challenge.[146] The importance of CD1-restricted T-cells for protection against Mtb infection in vivo is further substantiated by studies in transgenic mouse models expressing human CD1 molecules.[147] Adoptive transfer of MA-specific CD1b-restricted T-cells prior to Mtb challenge showed that these cells played a role in protective immunity against Mtb infection through TNF-α, IFN-γ, IL-2 production, and the expression of the cytotoxic granule release–associated membrane marker CD107a.[148] Optimized intracellular delivery of MA through polymeric micellar nanocarriers elicited potent CD1-restricted T-cells in mice, although it remains to be evaluated if this increased protection against Mtb.[149] Altogether, these studies show that lipid-reactive CD1-restricted T-cells are important players in immunity to, and in protection against, Mtb infection and that lipid-based vaccines, or whole-cell vaccines containing mycobacterial lipids, are promising strategies to improve immune protection against Mtb infection.

Structural analyses have shown that both the antigen-exposed polar cap and the distal hydrophobic antigen regions buried within the CD1 groove influence TCR recognition.[150-152] Simplified synthetic SGL variants have been proposed as potential vaccine components to prime CD1-restricted T-cells.[153] These and future developments for lipid antigen production, together with the understanding of TCR recognition requirements, will support the potential to administer these ligands as candidate vaccines targeting CD1-restricted T-cells against Mtb.

## 4.2 | (invariant) NKT-cells in immunity against Mtb

Type I or invariant NKT (iNKT) cells recognize α-galactosylceramide (α-GalCer) or phosphatidylinositol mannoside (PIM)[154] and are defined by conserved TCRα and TCRβ chains predominantly encoded by *TRAV10* joint to *TRAJ8* and *TRBV25*.[155,156] In contrast, type II NKT-cells include CD1d-restricted T-cells with diverse TCRs that are responsive to Mtb-derived phosphatidylglycerol, diphosphatidylglycerol, and phosphatidylinositol.[157]

Accumulating evidence highlights the importance of iNKT-cells for immune control of Mtb. Several studies reported reduced numbers of circulating iNKT-cells in active TB patients compared with LTBI and healthy controls.[158-160] Interestingly, circulating iNKT-cells in TB patients showed increased CD38, CD69, and HLA-DR expression compared with healthy controls, suggesting an activated phenotype.[48,160] Moreover, iNKT-cells from active TB patients, contrary to LTBI, showed a higher PD-1 expression, which was linked to reduced reactivity to α-GalCer and increased iNKT-cell apoptosis.[48,161] In NHP, which have conserved primate CD1 and TCR sequences,[162] an increased frequency of circulating iNKT-cells was linked to improved control of Mtb infection and disease.[163] In line with this, at TB diagnosis in humans, higher counts of NKT-cells, including cells that do not express an invariant TCR, strongly correlated with faster responses to antibiotic treatment.[164] Higher PD-1 expression on NKT-cells correlated with increased bacillary loads in TB patients, and PD-1 blockade was associated with increased NKT-cell survival and function.[165] Furthermore, NKT-cells isolated from pleural fluid from TB patients expressed not only Th1 and Th17 cytokines, but also IL-21 and CXCR5 upon PPD stimulation, and could induce B-cell function in vitro.[166] Altogether, these studies suggest that iNKT and NKT-cells could play an important role in the control of Mtb infection through distinct and complementary mechanisms.

An immunoregulatory role for iNKT-cells was suggested after BCG-infected NKT-cell deficient mice showed no differences in bacterial load but greater lung pathology compared with wild-type mice.[167] Although neither CD1d nor NKT-cell–deficient mice presented increased susceptibility to Mtb infection,[168,169] adoptive transfer of iNKT-cells demonstrated their contribution to the control of Mtb replication in vivo by recognition of infected macrophages.[170] In this study, iNKT-cell activation was shown to be TCR-independent but IL-12 and IL-18 induced, which triggered IFN-γ production. Similarly, induction of NKT-like cells in humans vaccinated with the subunit vaccine candidate H4:IC31 was associated with TCR-independent, cytokine-mediated activation.[171] In the absence of IL-12/IL-18, Mtb control by murine iNKT-cells depended on CD1d and granulocyte-macrophage-colony-stimulating factor (GM-CSF). GM-CSF is associated with control of intracellular mycobacterial proliferation in vitro and with protective immunity in mice,[172,173] and was shown to be induced by Mtb antigens in in vitro human studies.[174] Anti-microbial activity of α-GalCer–stimulated human iNKT-cell clones in vitro was associated with IFN-γ and granulysin production.[127]

Modulation of iNKT-cell responses to enhance protection against Mtb was explored through administering mycobacterial-derived lipids. Reduced bacterial loads and lung pathology were observed after Mtb challenge of guinea pigs vaccinated with PIM and SGL, lipids presented by CD1d and CD1b, respectively.[175] In mice, vaccination with α-GalCer–containing BCG led to enhanced DC maturation and increased $CD8^+$T-cell priming, which correlated with increased protection against Mtb compared with BCG alone, suggesting a role for iNKT-cells in bridging innate and adaptive immunity.[176] Similarly, α-GalCer–adjuvanted Ag85B and ESAT-6 subunit vaccines induced stronger $CD4^+$ and $CD8^+$T-cell responses accompanied by increased local and systemic protection against Mtb, compared with subunit vaccines or BCG alone.[177] α-GalCer also showed a protective effect against Mtb when administered, alone or in combination with antibiotics, shortly after infection, reminiscent of trained immunity effects likely through activating NKT-cells.[178-180] However, at later time points, the treatment had no benefits, possibly as a consequence of apoptotic or irresponsive NKT-cells, underscoring their

relevance as an early immune response component against Mtb infection, akin to the above observations for MAITs. Administration of a novel B-cell targeting vaccine expressing ESAT6 and loaded with α-GalCer showed both preventive and therapeutic effects in *M kansasii* infected mice, mediated by enhanced B-cell and $CD4^+$T-cell function.[181] Additional efforts are underway to design lipid-based vaccine strategies able to induce NKT-cell responses.[182]

## 5 | NK CELLS AND INNATE LYMPHOID CELLS (ILCS) IN IMMUNITY AGAINST MTB

### 5.1 | NK cells

NK cells have been observed within granulomatous lesions in humans, suggesting their contribution at the infection site.[183] A large multi-cohort study showed that circulating NK-cell frequencies were increased during LTBI, decreased during TB disease, and normalized after treatment, supporting a role for NK cells in Mtb infection control.[184] Importantly, longitudinal analysis identified that circulating NK-cell frequencies could indeed be a correlate of TB disease progression and an indicator of recovery after treatment. Moreover, functionally impaired NK-cell function was associated with reactivation of TB disease.[185] NK cells from LTBI showed downregulation of cytotoxic receptor NKp46 compared with uninfected controls.[186] In vitro, the expression of NK-cell–activating receptors was upregulated upon coculture with Mtb-infected cells and was associated with antigen-specific NK-cell–mediated lysis.[187,188] In BCG-vaccinated infants and children, NK cells were identified as one of the main sources of IFN-γ, highlighting their functional importance in the early immune responses against Mtb.[101] Although frequencies of peripheral NK cells remained unchanged, BCG vaccination in healthy adults led to increased production of proinflammatory cytokines IL-1β, IL-6, and TNF-α after in vitro exposure to Mtb or unrelated stimuli, reminiscent of trained immunity.[189] Importantly, memory-like antigen-specific NK cells isolated from TB patients' pleural fluid were shown to produce IL-22 in response to BCG and Mtb antigens, only when expressing CD45RO.[190] In a recent study, supporting the potential for vaccine-induced memory NK cells, BCG vaccination of mice led to the development of IL-21–dependent IFN-γ–producing memory-like NK cells, which were shown to provide protection against Mtb challenge.[191] Indeed, NK-cell memory phenotypes and their potential as vaccine target have been described in the context of diverse infectious diseases.[192] In adults revaccinated with BCG, NK-cell effector responses were long-lasting and IFN-γ production by these cells was reported to be dependent on IL-12 and IL-18 signaling.[193] Altogether, these studies highlight NK cells as potentially important players not only in the innate but also in trained innate memory immune responses to mycobacteria. However, in a recent study where intravenous administration of BCG was associated with protection against TB in NHP, the lack of innate cytokine production by PBMCs suggested that this response might be redundant for protection.[62]

BCG vaccination of mice induced NK-cell production of IL-22 and IFN-γ associated with reduced numbers of immunosuppressive Tregs and reduced bacterial burden after Mtb challenge.[194] NK cells were able to lyse extracellular mycobacteria through the release of cytotoxic granules[195] and to induce phagolysosomal fusion and Mtb growth control in infected phagocytes via IL-22.[196] NK cells activated by Mtb-infected monocytes could lyse expanded Tregs in vitro,[197] and multidrug-resistant TB was associated with decreased NK-cell function and elevated Treg expansion.[198] Moreover, NK cells induced TCRγδ T-cell proliferation via cell-to-cell contact or TNF-α, GM-CSF, and IL-12 signaling.[199] Furthermore, NK cells were shown to exert regulatory functions on $CD8^+$T-cells, enhancing their cytotoxicity via IFN-γ, IL-15, and IL-18.[200] In the absence of T-cell responses, IL-12–mediated NK-cell production of IFN-γ can play an important role in protection against Mtb in mice, a mechanism which might be relevant in patients with genetic or acquired immunodeficiencies.[201] CMV antigen presentation via the non-classical HLA class Ib molecule HLA-E has been shown to influence NK-cell expansion and to induce effector functions in NKG2C-expressing NK cells in vitro.[202] Differential recognition of HLA-E–presented peptide variants has been shown to modulate NK-cell adaptive immune responses, hinting to a possible mechanism through which protective NK cells could be induced.[203] This, together with the potential to target HLA-E–restricted T-cells discussed above, further underscores the importance of defining HLA-E–presented peptides and the requirements for TCR versus NKG2A/C recognition.

Altogether, NK cells present a functional spectrum of cells associated with protection to Mtb, via cytotoxic, regulatory, and memory activities in coordination with or independent from other immune cells (Table 1). The versatility for NK-cell induction through innate and adaptive mechanisms makes them an appealing cell target for mobilization via new vaccines.

### 5.2 | ILCs

ILCs lack rearranging antigen receptors typically found on T-cells and B-cells, and their function, mediated by cytokine stimulation, is important for mucosal immunity. Three different subsets are described based on their cytokine profiles: ILC1s share features with NK cells and respond to IL-12, IL-15, and IL-18 stimulation by producing IFN-γ; ILC2s are triggered by IL-25, IL-33, and thymic stromal lymphopoietin (TSLP) leading to a type 2 cytokine profile; and ILC3s, as the innate counterpart of Th17 cells, respond to IL-23 and IL-1β to secrete IL-17 and IL-22.[204] ILCs are involved in promoting protective immune responses to pathogens and in maintaining tissue integrity and homeostasis.[205] Circulating ILCs have been shown to be reduced in TB patients compared with healthy controls, and to be restored following treatment.[206] The accumulation of ILC3s in infected lungs, apparently recruited by CXCL13-CXCR5 signaling to localize to TB granulomas, was associated with Mtb control in humans and mice, mediated by IL-23–induced IL-17 and IL-22.[206] Transfer of ILC3s or IL-22 treatment reduced inflammation during Mtb infection resulting

in increased survival of type 2 diabetic mice, suggesting a role for ILC3s in protection against TB disease.[207] Supporting the possibility of vaccine-mediated induction of ILCs, IFN-γ–producing ILC1s and ILC3s were increased in lungs and lymph nodes of mice after BCG vaccination, a response which was more prominent after mucosal BCG administration compared with other routes.[208] Altogether, ILCs, and in particular ILC1s and ILC3s, are recently identified players participating in the human and mouse immune responses to TB, and future studies must delineate their role in vaccine-induced immunity (Table 1).

## 6 | TARGETING DURTS, NK CELLS, AND ILCS IN PREVENTIVE OR THERAPEUTIC VACCINATION AGAINST MYCOBACTERIA

DURTs, NK cells, and ILCs can help controlling Mtb and may synergize with classical Th1 T-cells and B-cells (Table 1). They may therefore be considered as interesting targets for vaccination to prevent infection or disease, or be mobilized via therapeutic strategies in treatment-resistant TB patients, for example, due to multidrug resistance.

### 6.1 | As vaccine targets to prevent infection (POI) or to prevent disease (POD)

Very recently, BCG revaccination in a phase-2 clinical trial showed efficacy in protecting Mtb-uninfected adolescents from acquiring sustained Mtb infection, suggesting POI by vaccination is possible.[209] Whole-cell vaccines, such as BCG, grant the possibility to target multiple cell populations simultaneously for induction of innate and adaptive immune responses, including DURTs, NK cells, and ILCs, through presenting alternative and diverse antigens. In contrast, subunit or virally vectored vaccines for TB have mostly focused on antigens inducing $CD4^+$T-cell responses essential to protection, among others through IFN-γ signaling,[210] although IFN-γ was not always a correlate of protection against Mtb infection and TB disease in clinical trials.[211,212] Recently, a phase 2 clinical trial with the subunit vaccine M72/$AS01_E$ reduced progression to TB by 50% in LTBI, although the lack of an adjuvant-alone control arm precluded the precise definition of the vaccine-induced mechanisms of protection.[213] Nevertheless, successful recent and future vaccine trials will allow the immunological dissection of protective responses and we strongly support assessment of the various DURT, ILC, and NK-cell populations in that context.

Advances in our understanding of the role played by DURTs, NK cells, and ILCs in protection against Mtb infection and TB disease now provide new and testable opportunities for vaccine and therapeutic targeting. Considering the effector, regulatory, and memory activities that have been identified for these unconventional immune cells, a whole-cell vaccine or combination of diverse antigens selected to induce an optimized and balanced response could represent an effective strategy. Nevertheless, the complex interactions occurring in vivo need to be addressed carefully. For instance, in addition to classical immune responses, BCG vaccination could induce trained immunity on NK cells, ILC-production of IFN-γ, and an array of DURT-mediated immune responses.[62,101,171,208,214,215] The delivery route of the vaccine is an important factor to consider, since intravenous BCG vaccination of NHP induced the transient recruitment of MAIT-cells and $Vγ9^+$ TCRγδ T-cells to the lung, and was associated with protection to Mtb challenge.[62] Pulmonary mucosal BCG delivery was also shown to prevent infection following repeated limiting-dose Mtb challenge, but DURT-cells were not investigated.[216] Other whole-cell vaccine candidates currently undergoing clinical testing are the genetically attenuated Mtb vaccine MTBVAC[217] and the recombinant BCG vaccine VPM1002.[218] However, their immunogenicity has been mostly evaluated in the context of Th1 and Th17 cytokine production. The upcoming efficacy trials for these vaccine candidates will likely contribute to the dissection of the immune cells involved in protection against Mtb infection and TB disease, including the role of DURTs, NK cells, and ILCs.

Alternatively to live whole-cell vaccines, non-replicating virally vectored vaccines are likely safer and can be designed to express a particular set of relevant antigens. The RhCMV-based vaccine, as discussed above, induced effector-memory responses and showed efficacy in preventing Mtb infection and TB disease in NHP.[27] Notably, HLA: classical versus non-classical (HLA-E) restriction of immune responses elicited by RhCMV-vectored vaccines could be selected based on the type of RhCMV strain used.[27] Similarly, $Vγ9Vδ2^+$ effector-memory T-cell induction by an attenuated HMBPP-producing *L monocytogenes* strain correlated with increased protection in Mtb-challenged NHP.[113] Further attempts to modify these vaccine delivery modalities and inserted antigens could help inducing increasingly diversified immune responses complementing and enhancing their vaccine efficacy.

Lipid and phosphoantigens inducing CD1-restricted and TCRγδ T-cells have been defined and synthesized and, in principle, are available to be included as vaccine components.[85,152,153] Of particular interest in this context is the design of efficient nanocarrier systems for intracellular delivery of antigens.[149] Moreover, efforts are underway to define novel HLA-E–presented peptides with optimal capacity to induce T-cells while simultaneously triggering a favorable NK-cell response.[219] Furthermore, MR1 ligands for MAIT-cell induction have also been identified, although their potential to induce protective responses in vivo remains to be elucidated. The lack of stability of MR1 ligands could be counteracted by modulating MAIT-cell responses through BCG vaccination,[62] via the microbiota,[78] or via TCR-independent mechanisms.[70] Similarly, iNKT-cells have been shown to be activated in mice through TCR-independent mechanisms mediated by IL-12 and IL-18 signaling,[170] and protective TCRγδ T-cells could also be induced with the combination of phosphoantigen and IL-2 in NHP,[117] supporting the use of cytokines for the induction or modulation of DURT responses against Mtb. Also, ILCs have the potential to be induced through targeted or bystander cytokine signaling.[206,208] The induction of protective iNKT-cells was also possible

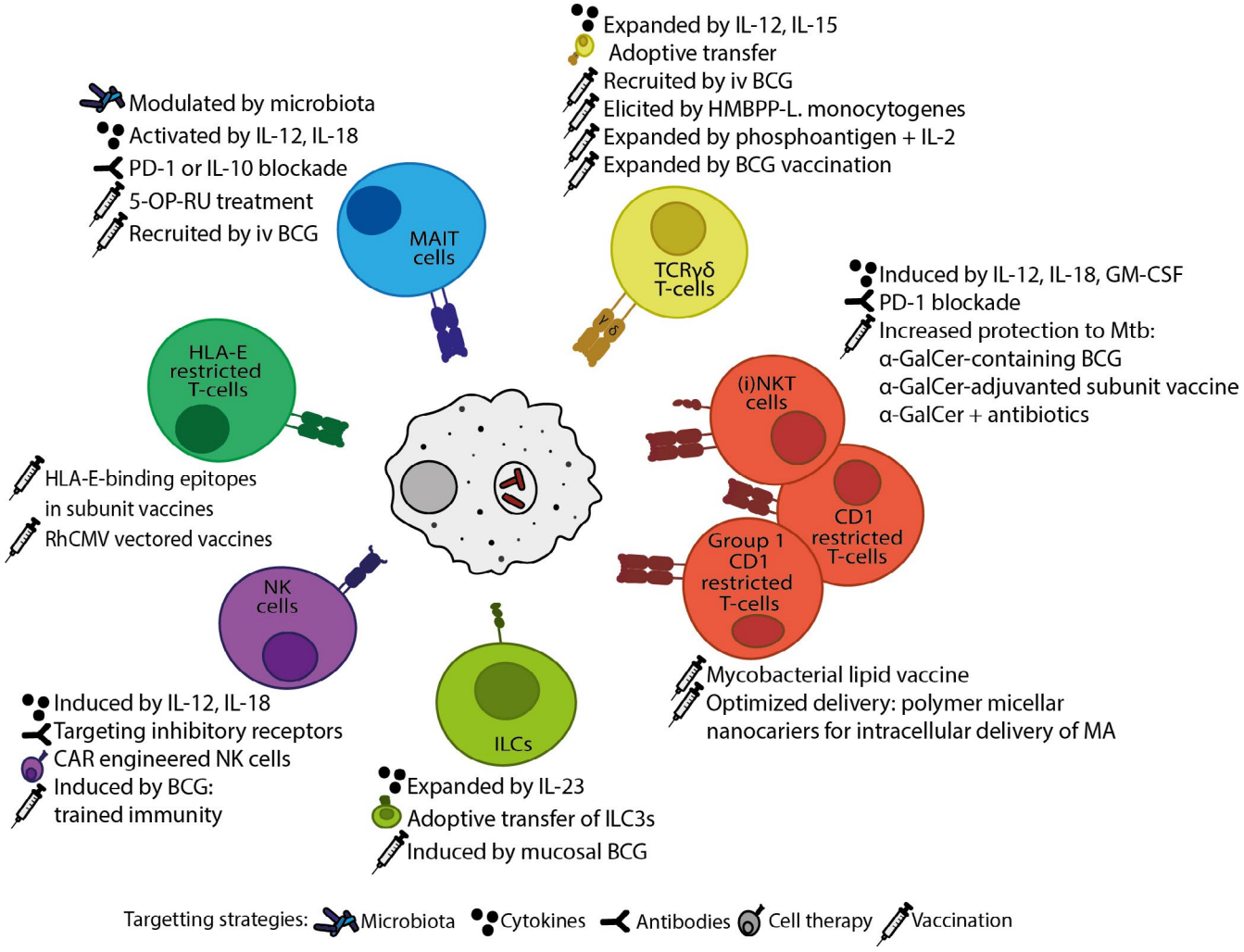


**FIGURE 1** DURTS, NK-cells and ILCs as unconventional targets in TB. DURTs, NK-cells and ILCs are important unconventional contributors involved in protective immunity in TB and other mycobacterial infections. Various potential approaches are being investigated to improve targeting DURTs, NK cells and ILCs for increased protection against Mtb-infection and TB disease. These include vaccine strategies ranging from classical BCG and modified whole cell vaccines to antigen-expressing viral vectors and subunit vaccines. Treatment with soluble agents such as cytokines and antibodies to increase the immune response can be complemented with the adoptive transfer of certain unconventional immune cells

through α-GalCer–adjuvanted subunit vaccine[177] or α-GalCer insert in a modified BCG vaccine,[176] further supporting the combination of antigens for targeting diverse immune responses. Although traditionally considered a component of the innate immune system, NK cells have been shown to have a memory phenotype inducible via trained immunity or antigenic triggering.[189,190] Opportunities to target protective NK cells in the context of TB could thus be inspired by the field of cancer immunology and HIV infection, where soluble agents, cytokines, and antibodies are used to modulate NK-cell function, as well as the design of chimeric antigen receptor (CAR) engineered NK cells.[220] Similar approaches are also being developed to target NKT-cells against tumors[221] and could potentially be expanded to target these and other DURTs in the context of TB.

Altogether, diverse strategies are currently being investigated to induce immune responses, which could contribute to protection against Mtb via targeting DURTs, NK cells, and ILCs in conjunction with more classical responses (Figure 1). The lack of polymorphism in antigen presentation molecules and the presence of innate-like features characteristic of these immune cell populations, along with their polyfunctional phenotypes in response to Mtb, represent important advantages and highlight their potential in vaccine and immunotherapeutic strategies.

## 6.2 | TB Treatment

Especially relevant in the context of multidrug-resistant and extensively multidrug-resistant TB are vaccine strategies designed to improve treatment outcomes in active TB, such as the liposomal vaccine candidate RUTI, based on detoxified fragments of Mtb-inducing polyantigenic immune responses.[222] Although knowledge on the efficiency of therapeutic vaccines is limited, the high

diversity in the reactivity of DURTs could potentially be exploited to induce protective responses to Mtb antigens that are particularly produced during active TB. This has been explored through vaccination in the context of MAITs in mice,[73] as well as through adoptive transfer of previously expanded TCRγδ T-cells in NHP.[114] Here too, the design of genetically engineered T-cells could provide a possibility for DURTs and NK cells to improve treatment outcomes in active TB. Moreover, α-GalCer administration in combination with antibiotics already has shown to improve outcome in Mtb-infected mice.[178]

## 7 | CONCLUDING REMARKS

Targeting DURTs, NK cells, and ILCs opens a range of possibilities to induce immune responses against Mtb infection and TB disease that complement each other and classical immune responses. These unconventional subsets are considered to contribute important, additional protective immunity in TB and other mycobacterial infectious diseases such as leprosy[159,223] and non-tuberculous mycobacteria infections, which can be difficult to treat. The ongoing challenge to define correlates of protection could be addressed, in part, by exploring the role of these unconventional responses in successful efficacy trials, and bridging human trials to detailed studies in NHP. Whether it is through whole-cell vaccines, vectored vaccines, subunit vaccines, or a combination of these and other approaches, future studies must consider the contribution of DURTs, NK cells, and ILCs to optimal protective immunity to mycobacterial infectious diseases, a plague to mankind and animals.

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### DATA AVAILABILITY STATEMENT

Data discussed were all retrieved from published literature as specified in the reference list.

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