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Spotlight

$\gamma\delta$ TCR Recognition of MR1: Adapting to Life on the Flip Side

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Nonclassical class I MHC-like molecules are ligands for several unconventional T cell populations.

Recently, Le Nours *et al.* identified human $\gamma\delta$ T cells recognising MHC-related protein-1 (MR1) via their T cell receptor (TCR). Also recognised by the $\alpha\beta$ -TCR of mucosal associated invariant T cells, MR1 interacts with specific $\gamma\delta$ -TCRs using strikingly diverse binding modes, suggesting fundamental differences in $\gamma\delta$ T cell recognition.

$\gamma\delta$ T cells, defined by their surface expression of paired γ and δ T cell receptor (TCR) chain heterodimers, have been retained throughout vertebrate evolution and play critical roles in host immunity in diverse settings, including infection, antitumour immunity, and immune regulation [1,2]. They are also of increasing therapeutic interest. Although it is widely accepted that unlike $\alpha\beta$ T cells, they do not recognise peptide-MHC molecules, the question of what antigens they do recognise via their TCR still remains substantially unresolved.

Since the discovery of $\gamma\delta$ T cells, diverse molecules have been proposed as candidate $\gamma\delta$ -TCR ligands [3]. While recent evidence has confirmed butyrophilin/butyrophilin-like (BTN/BTNL) family molecules as direct TCR ligands for $\gamma\delta$ T cell populations bearing specific TCR γ chain variable regions (either V γ 4/V γ 7 [4] or V γ 9 chains [5,6]), the mouse nonclassical class I MHC molecules T10 and T22 were the first $\gamma\delta$ -TCR ligands to be confirmed biochemically [7]. Since then, $\gamma\delta$ T cells capable of interacting via their TCR with the nonclassical class I MHC molecule CD1d have also been defined [8].

Recently, Le Nours and colleagues have made an important step forward by demonstrating a third category of nonclassical class I MHC molecule, MHC-related protein 1 (MR1), is also a target for $\gamma\delta$ -TCR binding [9]. Their study combines use of MR1-tetramer staining to identify MR1-binding $\gamma\delta$ T cell populations, surface

plasmon resonance (SPR) to assess direct $\gamma\delta$ -TCR/MR1 binding, and structural techniques to establish relevant binding modes. Their findings significantly advance our understanding of MR1 and may hold some fundamental lessons regarding $\gamma\delta$ -TCR recognition itself.

Adaptive $\gamma\delta$ T Cell Recognition of a Monomorphic Ligand

CD1d and MR1 are established recognition targets for defined $\alpha\beta$ T cell populations, namely invariant natural killer T cells (iNKTs) and mucosa associated invariant T cells (MAITs), respectively. Aligning with the monomorphic nature of these ligands, both iNKTs and MAITs express a highly restricted TCR repertoire and also exhibit distinct innate-like phenotypes relative to the bulk $\alpha\beta$ T cell compartment. Using MR1 tetramers, Le Nours *et al.* showed the situation is very different for MR1-binding $\gamma\delta$ T cells.

In most people, MR1-specific $\gamma\delta$ T cells comprised a low percentage (~0.1%) of $\gamma\delta$ T cells. Strikingly, their TCR repertoire was diverse, reflecting the TCR-diverse adaptive-like V δ 2^{neg} repertoire as a whole and chiefly focussed on the prevalent V δ 1 and V δ 3 subsets, combined with a broad range of V γ chains. Also, MR1-specific $\gamma\delta$ T cells phenotypically resembled the entire $\gamma\delta$ T cell pool. By contrast, the semi-invariant, innate-like V γ 9V δ 2 T cell subset, which bears a highly restricted TCR repertoire, was not a source of MR1-specific $\gamma\delta$ T cells. These features closely mirror those of CD1d-specific $\gamma\delta$ T cells (and $\gamma\delta$ T cells specific for the exogenous model antigen phycoerythrin), but contrast with properties of iNKTs and MAITs. Relative to $\alpha\beta$ T cells, $\gamma\delta$ T cell recognition of nonclassical class I MHC molecules may therefore be fundamentally skewed towards highly TCR-diverse, adaptive-like $\gamma\delta$ subsets, which are thought to bind a diverse array of ligands.

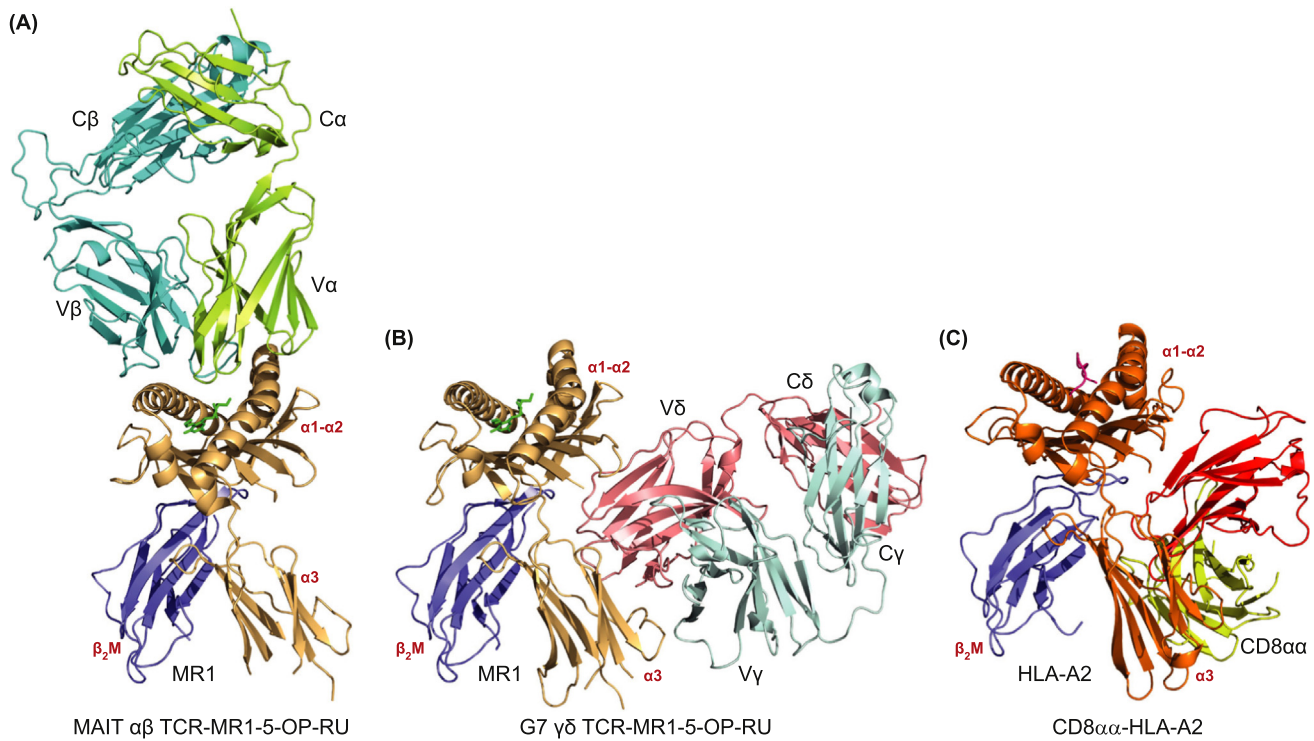
Whilst limited phenotypic analysis of MR1-specific $\gamma\delta$ T cells was carried out, their differentiation status was not defined. Addressing this question, highly relevant for adaptive compartments, would clarify if MR1-specific $\gamma\delta$ T cells reside within the T_{effector} subpopulation, consistent with *bona fide* MR1-directed adaptive T_{effector} responses, or alternatively within the T_{naive} subpopulation, which lacks effector capability and would be more suggestive of potential adaptive reactivities [10] yet to encounter MR1 *in vivo*. *In vitro* assays involving transduction of MR1-binding TCRs into Jurkat T cells showed that although CD69 upregulation was not always observed, MAP kinase/ERK kinase activation was universal, confirming a potential to support TCR triggering. Although

low levels of MR1-specific T cells were detected in most individuals, MR1-tetramer-positive cells were enriched in some individual samples, including in newly diagnosed coeliac disease and Merkel cell carcinoma. This finding suggests both TCR-diverse T_{naive} and clonally focused T_{effector} subpopulations may contribute to the MR1-specific $\gamma\delta$ T cell pool; the latter could contribute to physiological adaptive $\gamma\delta$ T_{effector} responses in some individuals. Future studies will no doubt shed light on these questions.

Diverse Modes of Antigen-Agnostic $\gamma\delta$ TCR Binding to MR1

Le Nours and colleagues also outlined the molecular basis of $\gamma\delta$ -TCR/MR1

interaction. SPR binding studies revealed MR1-binding $\gamma\delta$ -TCRs tested were largely ‘antigen agnostic’ and either entirely unaffected or only slightly impacted by the presence/absence of MR1-bound antigen, suggesting potential ‘inherent autoreactivity’ to MR1-expressing cells even in the absence of antigenic challenge. Although iNKT and MAIT TCR/ligand recognition has also been linked to ‘inherent autoreactivity’, this operates via binding modes apparently exclusively involving interaction of $\alpha\beta$ -TCR CDR loops with the $\alpha 1\alpha 2$ platform (Figure 1A). Moreover, conserved iNKT and MAIT TCR V-region usage and respective germline-encoded CDR1/2 loops provide a clear basis for such semi-invariant interactions, which appear literally and immunologically ‘restricted’



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Figure 1. Overview of the MAIT $\alpha\beta$ TCR-MR1-5-OP-RU, G7 $\gamma\delta$ TCR-MR1-5-OP-RU, and CD8 α -HLA-A2 Complexes. (A) Cartoon representation of the MAIT $\alpha\beta$ TCR-MR1-5-OP-RU complex (PDB ID: 4NQC): MR1, brown; β_2 -microglobulin (β_2M), blue; 5-OP-RU, green; α -chain, light green; β -chain, cyan. (B) Cartoon representation of the G7 $\gamma\delta$ TCR-MR1-5-OP-RU complex (PDB ID: 6MWR): MR1, brown; β_2M , blue; 5-OP-RU, green; V γ 9 chain, pale cyan; V δ 1 chain, salmon. (C) Cartoon representation of the CD8 α -HLA-A2 complex (PDB ID: 1AKJ): HLA-A2, orange; β_2M , blue; peptide, hot pink; CD8 α , red and yellow. Ig-like variable and constant domains for α , β , γ , and δ chains are indicated by V α , C α , V β , C β , V γ , C γ , and V δ , C δ respectively. Abbreviations: MAIT, mucosa associated invariant T cell; MR1, MHC-related protein 1; TCR, T cell receptor.

to the $\alpha 1\alpha 2$ platform of CD1d or MR1, allowing potential for discriminating presence/absence and nature of bound antigen.

By contrast, mutational analyses suggested that collectively, the MR1-binding $\gamma\delta$ -TCR pool was not limited to interaction with the upper-face of the $\alpha 1\alpha 2$ platform, but also contained TCR specificities recognising the membrane-proximal 'flip-side' of MR1, predominantly to the $\alpha 3$ domain. X-ray crystallographic analysis confirmed this highly novel binding mode. Importantly, 'flip-side' interaction was consistent with antigen 'agnosticism' and involved no contacts to upper-facing $\alpha 1\alpha 2$ helical platform residues, instead predominantly featuring $\alpha 3$ domain contacts, with additional interactions to the platform's underside (Figure 1B). Consistent with diverse V γ usage in the MR1-binding $\gamma\delta$ -TCR pool, interaction was dominated by V δ -mediated contacts. Moreover, while some CDR1 δ -mediated involvement was evident, V δ interactions involved critical hydrophobic contacts formed by CDR3 δ residues, consistent with only a small proportion of the extremely diverse V $\delta 1$ TCR repertoire satisfying the molecular criteria for MR1 recognition. This mode resembled CD8 $\alpha\alpha$ /class I MHC recognition (Figure 1C), which itself was likened to antibody/antigen interaction [11]. These observations confirm that $\gamma\delta$ T cell recognition of MR1 is indeed fundamentally different to CD1d/MR1-restricted recognition by semi-invariant iNKTs and MAITs.

In summary, the identification of MR1-binding $\gamma\delta$ T cells is a significant advance for both MR1 and $\gamma\delta$ T cell biology and

should be applauded. By contrast to iNKTs and MAITs that now have established contributions to immune regulation, including in diverse models of infection/disease, the physiological role and importance of $\gamma\delta$ T cells that recognise nonclassical class I MHC molecules has remained largely unclear since their initial identification 20 years ago. In this context, the immunobiological meaning and relevance of antigen-agnostic recognition of MR1 by $\gamma\delta$ T cells is currently unclear. Moreover, future studies should consider the parallel and nonmutually exclusive possibilities that MR1 interactions with $\gamma\delta$ -TCRs either contribute to physiological adaptive $\gamma\delta$ T cell effector immune responses, or alternatively in some cases largely represent potential autoreactivities. In this second scenario, the presence of MR1-specific cells may reflect the fundamental potential of the adaptive $\gamma\delta$ -TCR repertoire to recognise diverse self-antigens, from which particular autoreactive TCR specificities may be selected to differentiate into T_{effector} cells to support adaptive $\gamma\delta$ T cell immunosurveillance following relevant immune challenges. Given the recent finding that MR1-directed $\alpha\beta$ -TCR alloreactive recognition of an antigenically altered form of MR1 can mediate broad antitumour responses [12], it is tempting to speculate on the potential relevance of $\gamma\delta$ -TCR/MR1 interactions in such settings, particularly given established *in vitro* antitumour capabilities of $\gamma\delta$ T cells. The study by Le Nours and colleagues is a fundamental step forward that should pave the way for future studies to address such fascinating questions.

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