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SPOTLIGHT

MR1: An unconventional twist in the tail

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MR1 is a conserved molecule that binds microbial vitamin B metabolites and presents them to unconventional T cells. Lim and colleagues (2022. *J. Cell Biol.* https://doi.org/10.1083/jcb.202110125) uncover the role of AP2 in ensuring MR1 surface presentation, which relies on an atypical motif within the MR1 cytoplasmic tail.

A standard view of the immune system has a division between innate responses (rapid but relatively non-specific) and adaptive responses (specific to the pathogen or vaccine, but slower to initiate). However, between these innate and adaptive cells there is a pool of "innate-like cells," notably unconventional T cells.

The definition of unconventionality is originally based on the target of recognition. Conventional T cell responses are mediated by T lymphocytes, which bear rearranged antigen receptors (T cell receptors [TCRs]), comprising a unique alpha and beta chain that are triggered by Major Histocompatibility Complex (MHC) Class I or II molecules presenting peptide antigens (1). In contrast, unconventional T cells recognize a wide range of antigens, typically presented by highly conserved MHC Class 1b molecules. Such antigens include a range of lipid antigens presented on CD1 molecules, and an intermediate metabolite of the riboflavin synthesis pathway—5OPRU—presented on MR1 (MHC related antigen 1) molecules. The latter is recognized by a population of unconventional cells known as mucosal-associated invariant T cells, or MAIT cells, which are abundant in humans, especially in tissues such as the liver (2, 3).

Along with an unconventional choice of antigen, the group of unconventional T cells share other features. Typically, they use a restricted set of TCRs, allowing them to be readily identified. Indeed, this is how MAIT cells were first discovered (4), since they use a single TCR alpha chain (Vα7.2 in humans) with a limited set of beta chains. Unconventional T cells also display a distinct phenotype and transcriptome (5). Thus, in addition to many biologic features common to $CD8^+$ cytotoxic T cells, they also show an unusually strong sensitivity to innate cytokines (such as interferons) and a much wider range of effector functions. MAIT cells, for example, exhibit quite distinct responses according to whether they receive TCR signals or cytokine triggering (or both). This unconventional responsiveness facilitates MAIT cell functions ranging from cytotoxicity and protection against microbes on the one hand to tissue repair on the other (6). Ensuring MAIT cells are sufficiently, but not overly, stimulated to carry out these functions must be under careful control.

Unlike conventional MHC Class 1a molecules presenting peptides, the expression of MR1 on the cell surface is highly restricted (7). Despite certain similarities—both MR1 and MHC Class I bind beta-2-microglobulin (β2M), for example—the mechanisms which govern MR1-antigen loading and how loaded complexes are regulated at the cell surface are still not completely understood. This is the issue that Lim and colleagues set out to explore (8).

The authors utilized a gene knockout screen, where cells treated with a CRISPR-Cas9 loss-of-function library were first exposed to antigen for 4 h and then incubated in antigen-free media for 8 h. Cells with high MR1 surface expression after the antigen-free chase period must have lost a gene essential for MR1 internalization. Remarkably, this approach revealed only one significant hit, AP2A1, one of four subunits of the AP2 adaptor complex, involved in the early steps of clathrin-mediated endocytosis. Specific deletion of AP2A1 in two different antigen-presenting cell lines reduced MR1 internalization and recycling and could be partially rescued upon re-expression of the protein.

So how exactly does AP2A1 recognize MR1? The CD1 family of antigen-presenting molecules are similarly internalized by AP2 complexes based on sequences in their cytoplasmic tail. Specifically, the presence of either a tyrosine- or leucine-based YXXΦ motif dictates AP2 binding and intracellular sorting (9). To determine if the cytoplasmic tail of MR1 contains any such motif, the authors compared sequences from 60 different mammalian species. They found evidence of a highly unusual but highly conserved tyrosine motif, where the typical bulky hydrophobic residue was replaced with a threonine residue. To test the importance of this motif for AP2 binding, the authors constructed a series of mutations within the cytoplasmic tail of MR1. Only mutation of tyrosine to alanine (Y313A) within the putative tyrosine motif significantly reduced MR1 internalization, akin to the phenotype observed with deletion of AP2A1. The significance of this interaction was then explored using a modified MR1 antigen analog (MAgA-TAMRA), as well as

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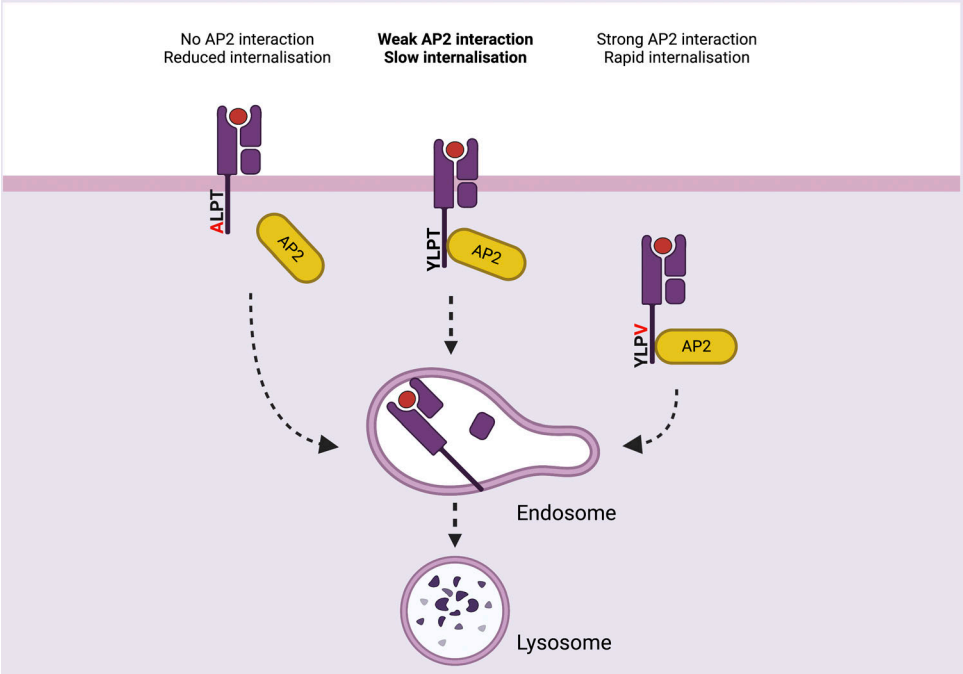

Figure 1. **MR1 internalization from the cell surface by AP2 is defined by the tyrosine motif in its cytoplasmic tail.** Mutation of the tyrosine residue (left) ablates the MR1-AP2 interaction, leading to reduced MR1 internalization. Mutation of the threonine residue to a more hydrophobic amino acid (right) results in a strong interaction and rapid internalization. The atypical motif normally present in the MR1 tail leads to a weak AP2 interaction and slow internalization (center, bold), likely tuned for optimal immune activation. Once internalized, the MR1-β2M-antigen complex disassociates in endosomes and is then degraded in lysosomes. Created with BioRender.com.

Jurkat cells expressing the MAIT TCR (Jurkat.MAIT). In both cases, deletion of AP2A1 or expression of the Y313A mutant MR1 resulted in prolonged antigen presentation due to inefficient MR1 internalization (Fig. 1).

The impact of the atypical threonine residue within the tyrosine motif was then addressed by swapping the MR1 tail with that of CD1d and also specifically mutating the residue to make it more hydrophobic (T316V). Both changes resulted in increased MR1 internalization and recycling, which decreased levels of antigen presentation. This is presumably due to a stronger interaction between MR1 and AP2. Thus, the tyrosine motif acts as a molecular switch, dictating the length of time that MR1 remains on the cell surface for sufficient antigen presentation. The high-affinity interaction between AP2 and CD1d results in internalization rates of around an hour, whereas the lower affinity interaction between AP2 and MR1 leads to much slower internalization, taking several hours.

Why might an extended period of antigen presentation be important for MR1 in particular? Other antigen-presenting molecules, once internalized, are able to sample antigen from endocytic compartments and recycle back to the cell surface for presentation. The authors clearly demonstrate that this is not the case for MR1. MR1-β2M complexes are unstable at the low pH found in endosomes and lysosomes and so the majority of MR1 antigen loading occurs in the ER, with only a small proportion recycling back to the cell surface. Thus, a low-affinity interaction prolongs MR1-antigen surface expression before internalization and subsequent degradation.

So what are the implications of these findings for MAIT cells? One issue for MR1/MAIT biology is that the ligands for such cells are continuously generated by commensal microorganisms. The MR1 ligand, 5OPRU, is relatively unstable but still able to survive transport from the sites of microbial colonization such as the skin and gut and can, for example, load cells in the thymus and liver (10). The triggering of MAIT cells must therefore be tuned in vivo such that they are activated appropriately. Limiting the level of loaded MR1 reaching the surface but keeping it there longer might fit well with the proposed role of such cells in long-term tissue homeostasis. Further in vivo studies could explore this idea.

There is another fascinating aspect of MR1 mediated antigen presentation—its ability to present alternative ligands linked to cellular transformation. Although the nature of these ligands is not yet well defined, specific "MR1-T" cell clones which recognize diverse cancer cell lines through MR1 have been grown (11). How such ligands are loaded and how this recycling pathway may impact on immune targeting are questions now also relevant to cancer therapies as well as infectious diseases. So expect more twists in the tale as the unconventional nature of MR1 biology is further explored and revealed.

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