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RESEARCH HIGHLIGHT

Mucosal associated invariant T cells: Don't forget your vitamins

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T lymphocytes express clonal receptors, called T cell receptors (TCRs), which specifically recognize antigens presented in combination with major histocompatibility molecules (MHC). To date, T cell antigens can be broadly categorized into two classes: peptides and lipids. A recent paper published in *Nature* by Kjer-Nielsen and colleagues reveals that a unique population of T lymphocytes expresses TCRs that recognize a completely new and unexpected class of antigens, vitamin metabolites.

The immune system has evolved a variety of defense mechanisms against foreign pathogens, including both innate and adaptive processes that work in concert to eliminate potential threats. The innate arm of the immune system includes cells that express a variety of non-polymorphic, generic receptors, which recognize structurally conserved molecules derived from microbes. In contrast, the adaptive arm includes cells that express clonally distributed variable receptors generated through somatic rearrangements of gene segments, which recognize specific antigens derived from pathogens. Engagement of these receptors at the surface of lymphocytes by their specific antigens results in clonal division and the production of cellular mediators. The variable receptors are immunoglobulin, expressed by B lymphocytes, and the αβ T cell receptor (TCR), expressed by the vast majority of T lymphocytes.

In contrast with immunoglobulins, which can recognize virtually any antigenic structure, αβ TCRs recognize antigens that are displayed by antigen-presenting molecules, such as the ones encoded by the major histocompatibility complex (MHC). MHC class I and class II are polymorphic molecules that present a multitude of antigens in the form of peptides derived from pathogens. However, it is now clear that a significant fraction of T lymphocytes bear αβ TCRs that do not recognize conventional MHC molecules plus peptides but instead are directed at what has been labeled as "non-classical" MHC-like molecules. These "non-classical" MHC-like molecules are often encoded in the genome outside of the MHC locus itself and display little to no polymorphism. As such, a unique role in antigen presentation is usually expected from these "non-classical" MHC-like molecules. For example, H2-M3 molecules have the unique capacity to present bacteria-derived N-formylated peptides [1], while members of the CD1 family, which includes the well-studied CD1d molecule, present lipid antigens [2].

While CD1d plus lipid complexes can be recognized by a variety of lymphocytes bearing different αβ TCRs, they are also the target of a unique innate-like T lymphocyte population called natural killer T (NKT) cells. The NKT TCR is somewhat of an anomaly in the world of classical αβ TCRs in that is formed through the usage of a restricted set of gene segments. The α chain of the NKT TCR is always comprised of a single canonical rearrangement between the *TRAV11* and *TRAJ18* gene segments in mice (or the orthologs genes *TRAV10* and *TRAJ18* in human), which pairs with a limited set of Vβ segments. The NKT TCR has been shown to recognize a variety of self and foreign lipids presented by CD1d and its engagement at the surface of NKT cells leads to a rapid and diverse cytokine secretion storm. As such, NKT cells have been implicated in the regulation of a multitude of immunological processes, including infections, cancer, and autoimmunity [3].

Another subset of T cells bearing a restricted αβ TCR repertoire was recently identified [4, 5]. Due to their preferential localization in the gut lamina propria, these cells were deemed mucosal-associated invariant T (MAIT) cells [6]. Their 'semi-invariant' TCRα chain is composed of a limited set of rearrangements between the *TRAV1* and the *TRAJ33* gene segments, which pair with a limited set of Vβ chains. The generation of a monoclonal antibody directed at the human TRAV1 chain allowed for the enumeration and tracking of MAIT cells. Surprisingly, it was found that MAIT cells can constitute up to 10% of human peripheral blood T cells and up to 40% of human liver T cells [7].

The TCRs expressed by MAIT cells were shown to recognize the MHC-related protein 1, MR1, a very intriguing non-classical MHC class I molecule in its infancy of characterization [6]. MR1 is encoded outside of the MHC locus in human, mouse and rat, and shows 90% sequence identity in its putative ligand-binding domains (α1/α2) between the human and the mouse, which far exceeds the 70% similarity shared by this region of human and mouse classical MHC class I molecules. The strict conservation of both MR1 and MAIT

cells among mammalian species, as well as the important proportion of MAIT cells within the human T lymphocyte population, are all suggestive of stringent evolutionary pressure for important function(s) fulfilled by MAIT cells. In support of this hypothesis, it was shown that MAIT cells are activated by cells infected with various strains of bacteria and yeast in both human and mouse [8, 9]. This activation required cognate interaction between the invariant TCR and MR1, which was proposed to present a bacteria-derived ligand. In this way, these lymphocytes can rapidly sense and help fight off microbial infections. However, the exact nature of this putative bacteria-derived ligand has remained elusive.

In a recent issue of *Nature*, Kjer-Nielsen *et al.* [10] shed some new light on the nature of the MAIT antigens. Owing to the fact that, in general, MHC class I molecules are extremely unstable unless they have engaged a ligand, Kjer-Nielsen *et al.* found that refolding of MR1 in the presence of vitamin-containing solutions substantially increased their yield of refolded MR1 proteins. Taking advantage of this finding, they further refined their candidate ligands and identified the presence of the folic acid (vitamin B9) metabolite, 6-formyl pterin (6-FP), bound to MR1. Further, they provided the first crystal structure of the MR1 protein in complex with 6-FP, thereby revealing how the MR1 antigen-binding groove appears ideally suited to present small organic compounds. Interestingly, 6-FP was found in the MR1 antigen-binding cleft where it sits horizontally with no residues extending over the α helices for potential recognition by a TCR. Indeed, the authors showed that although 6-FP can clearly be presented by MR1 molecules, it is non-stimulatory for MAIT cells.

The authors were also able to refold MR1 in the presence of culture supernatant from *Salmonella typhimurium*, a bacterial strain known to stimulate MAIT cells. Mass spectrometry analysis of MR1-complexed ligands revealed metabolites from the riboflavin (vitamin B2) biosynthesis pathway. The riboflavin metabolites are structurally similar to 6-FP, but possess an extra ribityl moiety, which is postulated to extend up into the groove of MR1 and be accessible for TCR recognition. In support of this hypothesis, the riboflavin metabolites are able to stimulate human MAIT cells as well as Jurkat cells engineered to express three different human MAIT TCRs.

These findings have important implications, not only for the emerging field of MAIT cell biology but also for immunologists in general. In addition to peptides and lipids, the immune system contains T cells that have the ability to recognize and survey a third class of antigens: vitamin B metabolites (Figure 1).

Mammals, including humans, have lost the capacity for *de novo* synthesis of B vitamins and rely on diet as well as intestinal bacteria and yeast species that can synthesize them for their acquisition via intestinal absorption. B vitamins are essential components of many cellular processes, with many functioning as precursors for enzyme cofactors or playing the role of coenzymes that carry chemical groups or electrons between molecules. Importantly, riboflavin (vitamin B2) itself, which is found in all mammalian cells, did not stimulate human MAIT cells, but its metabolic precursor, 6,7-dimethyl-8-ribityllumazine, did. These results suggest that the role of MAIT cells might be to survey microbial infections or overgrowth at mucosal sites by sensing the overall quantity of riboflavin metabolites in an MR1-restricted manner. In support of this idea, the authors noted that bacteria and yeast species that were found to stimulate MAIT cells all possess a complete riboflavin synthesis pathway, while other non-stimulatory species did not have this ability.

The findings by Kjer-Nielsen *et al.* raise many new interesting and intriguing questions. The study highlights that MR1 molecules can present both vitamin B2 and B9 metabolites, yet only vitamin B2 metabolites can stimulate MAIT cells. These results raise the possibility that several different metabolites might compete for MR1 binding and thereby modulate the activation of MAIT cells. To date, the mechanisms of antigen presentation by MR1 molecules remain largely unexplored.

Like conventional αβ T cells that undergo positive selection by self-peptide-MHC complexes in the thymus, MAIT cells also develop in the thymus, where they must recognize MR1 molecules, presumably loaded with antigens, for proper development [6, 11]. Are these antigens really "self" or are they, as the absence of MAIT cells in germ-free mice could perhaps suggest, metabolic products derived from the microbiota? Although riboflavin transporters have been identified [12], it remains unclear whether and how its metabolites might be transported throughout the organism. Furthermore, certain clones of MAIT cells can detect non-infected MR1-expressing antigen-presenting cells (APCs), suggesting that some MAIT TCRs might have a dual specificity for both microbe-derived metabolites as well as APC-derived, or media-provided, antigen(s). These results imply that perhaps other antigenic structures distinct from vitamin metabolites might exist for MAIT cells. Identification of the antigen(s) that are involved in intrathymic MAIT cell selection will certainly remain a central goal in the future.

Finally, the preferential localization of MAIT cells in the lining of mucosal surfaces and their protective role in several infections [8, 9, 13, 14] open new avenues for the development of vaccine strategy that specifically targets MAIT cells but also call for the exploration of a potential role of MAIT cells in mucosal disorders such as Crohn's disease and ulcerative colitis.

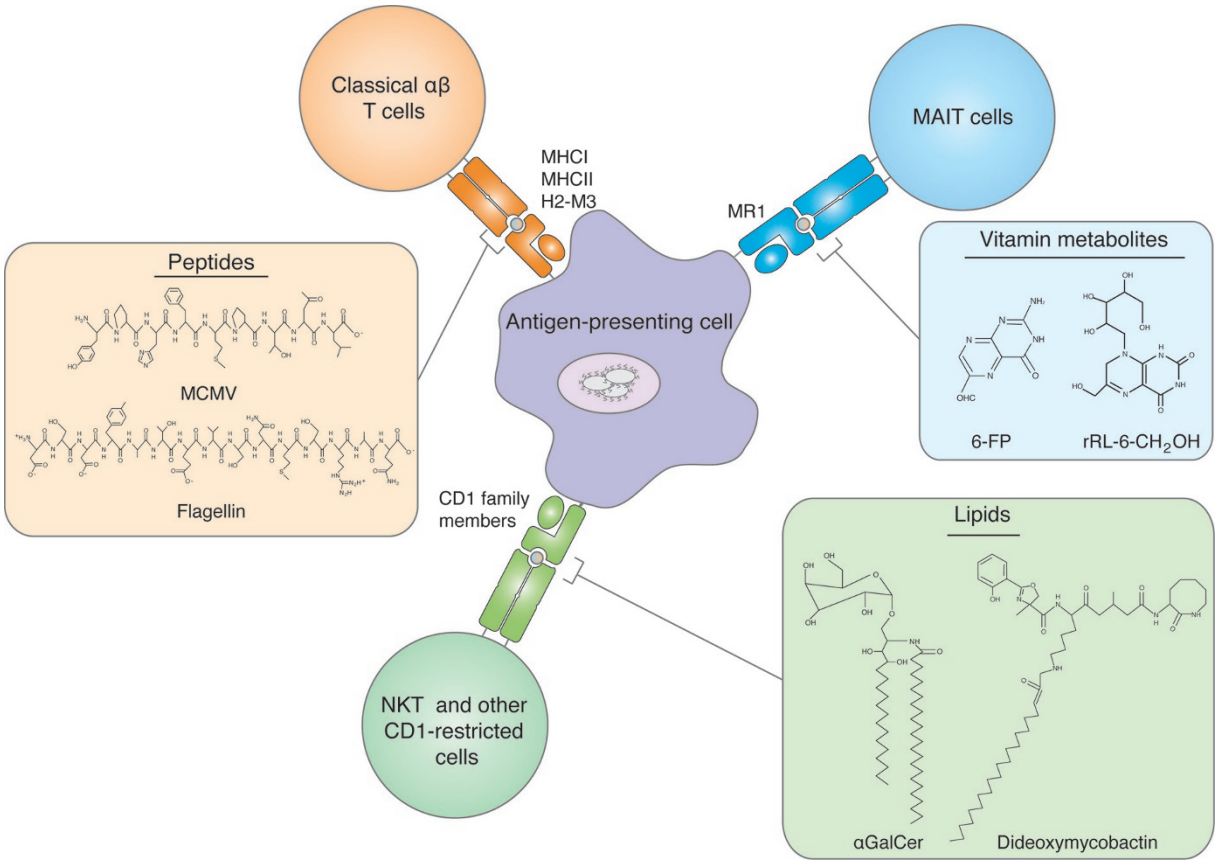

Figure 1 Three broad classes of ligands recognized by TCRs: peptides, lipids, and vitamin metabolites. Examples of each class of ligands are shown. Peptides from MCMV and flagellin are presented by MHC class I or class II to classical αβ T cells, respectively. Vitamin metabolites are presented to MAIT cells by MR1. The lipids, α-galactosylceramide (αGalCer) and dideoxymycobactins presented to NKT cells and CD1a-restricted T cells, respectively.

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