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INSIGHTS

An unconventional purine connection

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Xu et al. (https://doi.org/10.1084/jem.20240354) define NAD-induced cell death via purinergic P2RX7 receptor in type 1 unconventional T cells, particularly intrahepatic MAIT cells that are pivotal in liver homeostasis. Therefore, P2RX7 is a potential target to modulate unconventional T cells in immunopathological conditions and cancer.

The mechanisms that regulate the functional shaping of the immune response against the multitude of pathogenic threats arising in different tissues are incompletely defined. Unconventional T cells lay at the intersection between the innate and adaptive immune system and comprise a variegated group of cell subsets, including γδ T, natural killer T (NKT), and mucosal-associated invariant T (MAIT) cells, recognizing non-polymorphic self or evolutionarily conserved microbial antigens. In recent years, it has become clear they have a crucial role in ensuring barrier integrity at mucosal surfaces and complementing conventional T cells in tissue protection and healing. However, the factors controlling the responsiveness and functional differences among the distinct subsets of these cells are poorly characterized. A new study from the *Journal of Experimental Medicine* unravels the peculiar sensitivity of murine Tbet$^+$ "type 1" MAIT, γδ T, and NKT cells, particularly in the liver, where they comprise up to 50% of T cells, to a cell death-inducing pathway referred to as nicotinamide adenine dinucleotide (NAD)-induced cell death (NICD). This form of cell death is dependent on ADP-ribosylation and activation of the purinergic receptor P2RX7 by the ecto-ADP-ribosyltransferase ARTC2.2 (ART2) (Adriouch et al., 2008). P2RX7 is a non-selective cationic channel activated by extracellular ATP (eATP). The receptor is characterized by dual gating whereby low concentrations of eATP induce small-amplitude currents as compared with stimulation by saturating agonist concentrations, which results in high-amplitude biphasic currents reflecting the opening of a cytolytic pore and cell death (Khadra et al., 2013). Blocking ART2 or P2RX7 during ex vivo cell isolation procedures spares mouse ART2-expressing T cells from cell death and allows significantly improved cell recoveries of NICD-sensitive cells (Rissiek et al., 2014). By applying this knowledge, Xu et al. (2024) identified a so far elusive MAIT cell subset co-producing IFN-γ and IL-4 in the liver and demonstrated that P2RX7 activation primarily depletes T cells co-producing IFN-γ and IL-4.

The liver is the largest organ in our body and performs hundreds of vital functions. The portal circulation constitutes a roadway for gut-derived dietary and microbial antigens, thereby imposing tight regulation of the local immune response to ensure protection against pathogens and tolerance to innocuous antigens to avoid unnecessary tissue damage. eATP and NAD are endogenous harbingers of cell injury and inflammation; the exquisite sensitivity of IFN-γ and IL-4 co-producing T cells to NICD suggests they are poised for regulation by purinergic signaling. Importantly, the liver is exposed to metabolites generated by the intestinal microbiota, among which eATP can be detected in the portal vein blood (Proietti et al., 2019). It is plausible to hypothesize that microbiota-derived eATP condition immune cells in the liver via purinergic receptors and act as a signaling molecule in the gut-liver axis via P2RX7. An important aspect to be addressed in future studies is whether the ecto-ADP-ribosyltransferase enzymatic activity is involved in controlling unconventional and possibly other T cell subsets in humans. Albeit the study by Xu et al. (2024) show robust P2RX7 expression in unconventional as compared with conventional T cells and P2RX7 upregulation in human liver resident cells, there is no evidence for cell-intrinsic NICD in human T cells. Human γδ T and MAIT cells express P2RX7, and γδ T cells are susceptible to eATP-mediated cell death. However, ART2 is expressed in mouse T cells where it mediates NICD by catalyzing ADP-ribosylation of P2RX7 in cis, but not in human T cells. The presence of premature stop codons in the human

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ART2 gene precludes its expression, whereas other members of the ART family, including ART1, ART3, ART4, and ART5 are expressed (Haag et al., 1994). Although no direct evidence exists for NICD mediated by ADP-ribosylation of P2RX7 in human T cells, membranous expression of ART1 on human lung adenocarcinoma was associated with reduced tumor infiltration by P2RX7$^+$ CD8 T cells, suggesting it could mediate NICD in trans (Wennerberg et al., 2022). An analogous mechanism could control P2RX7 activity in human unconventional T cells in addition to eATP.

Gating of P2RX7 induces metalloprotease-mediated ectodomain shedding of plasma membrane proteins, thereby contributing to coordinating the T cell response. ART2 is a GPI-anchored protein, which is a target of P2RX7-triggered ectodomain shedding by activation of the membrane-bound metalloprotease ADAM17. This phenomenon results in refocusing the target specificity of ART2 from the plasma membrane to secretory proteins (Menzel et al., 2015). A notable consequence is ADP ribosylation of IFN-γ and inhibition of IFN-γR activation (Menzel et al., 2021). Therefore, purinergic stimulation of liver resident ART2-expressing T cells may rapidly result in limiting the signaling function of IFN-γ. Whether a similar regulatory pathway independent from ART2 is triggered by P2RX7 stimulation of human T cells remains to be investigated.

Xu et al. (2024) show that cognate antigen encounter in vivo results in rapid loss of ART2 by activated MAIT1 and NKT1 cells, which was associated with resistance to NICD. This phenomenon is reminiscent of P2RX7 downregulation observed in T follicular helper cells (Proietti et al., 2014) and CD8$^+$ tissue-resident memory T cells (Stark et al., 2018) to privilege the response of antigen-specific versus bystander T cells in an inflammatory environment. In addition, P2RX7-mediated cell death was shown to avoid the expansion of potentially pathogenic T cells (Faliti et al., 2019). Although P2RX7 was not downregulated in unconventional T cells, resistance to NICD by ART2 loss would result in the selection of antigen-specific cells and elimination of "inappropriate" bystander cells. Whether the persistent expression of P2RX7 renders these cells still susceptible to cell death induced by eATP or an additional regulatory layer controls P2RX7 expression upon persistent antigen stimulation requires further investigation. Since P2RX7 is endowed with dual gating that is graded by eATP concentrations, the dichotomy between NICD and eATP responsiveness could set a checkpoint for early cell death induced by NAD and modulation of subsequent responses by eATP levels in an evolving microenvironment. At the moment, this double-layered control of purinergic signaling would not be applicable to human T cells due to the lack of evidence for an ecto-ADP ribosyl transferase activity. Nevertheless, it would be interesting to address whether antigen stimulation of human unconventional T cells regulates P2RX7 expression levels and controls susceptibility to cell death by eATP.

The tumor microenvironment (TME) is characterized by high concentrations of eATP. Studies addressing the role of MAIT cells in tumors generated divergent results. This was probably due to the different context-dependent contribution of MAIT cells to tumor immunity, conditioned by cancer type, site, and stage. In vivo antigen-specific activation of MAIT cells in tumor-bearing mice resulted in their expansion and type 1 polarization with increased IFN-γ production and expression of cytolytic effector molecules, leading to enhanced control of tumor growth (Petley et al., 2021; Ruf et al., 2021). These data suggested that boosting MAIT cells could be exploited to treat tumors of various origins. The characterization of MAIT cells at major body sites within individual human donors revealed the functional heterogeneity across tissues as well as the specialization of distinct regulatory and effector profiles. In the intestine, a resident MAIT cell population was characterized by a CD27^{low}CD39^{high} phenotype and the production of regulatory as well as protective cytokines, including IL-10, IL-17, and IL-22. Notably, CD39 (ectonucleoside triphosphate diphosphohydrolase-1, ENTPD1) is a plasma membrane, eATP-degrading enzyme known to promote immunosuppression and limit immunopathological damage of tissues in inflammatory conditions. As expected, MAIT cells were particularly enriched in the liver, but in contrast with the intestinal cell pool, they were characterized by the high expression of CD56 (a marker of NK cells) and a gene signature reflecting enhanced competence for activation and cell killing (Kammann et al., 2024). The features of intestinal and intrahepatic MAIT cells hint at a fundamental role of these cells in tissue homeostasis; thus, MAIT cells might constitute a therapeutic target in inflammatory and neoplastic conditions. In hepatocellular carcinoma (HCC), MAIT cells contributed to antitumor immunity. However, they were characterized by impaired tumor infiltration as well as increasing dysfunction/loss of cytotoxicity within the TME likely due to interaction with PD-L1 expressing tumor-associated macrophages. Interestingly, in vivo MAIT cell activation synergized with anti-PD-1/PD-L1 based immune checkpoint blockade in controlling orthotopic HCC in mice (Ruf et al., 2023).

Purinergic signaling is an ancestral modality of sensing environmental and cell-intrinsic clues that are diffused in all kingdoms of life. The study by Xu et al. (2024) unravels P2X7R as a receptor conditioning the function of unconventional T cells, particularly intrahepatic MAIT cells. The existence of a large array of drugs, biologics, and biotherapeutics able to inhibit or stimulate P2X7R activity paves the way to novel therapeutic approaches in inflammatory and neoplastic conditions of the liver. The pleiomorphism and diffusion of P2X7R in a multitude of different cell types constitute a caveat to be carefully considered when designing therapeutic strategies targeting the receptor. Nevertheless, the relevance of P2X7R signaling in MAIT cells suggests it could be targeted in vivo as well as ex vivo in cell therapy approaches to shape and/or enhance the possible therapeutic efficacy of this intriguing cell population.

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