JEM Journal of Experimental Medicine

INSIGHTS

INPP4B ensures that ILC1s and NK cells set up a productive home office

Zi Yan Chen[1] and Arthur Mortha[1]

In this issue of *JEM*, Peng et al. (https://doi.org/10.1084/jem.20230124) identify inositol polyphosphate 4-phosphatase type II (encoded by *Inpp4b*) as an important enzyme for tissue-resident ILC1 and NK cell survival, signal transduction, and anti-tumor immunity.

Innate lymphoid cells (ILCs) are a heterogeneous population arising from a common innate lymphocyte precursor by integrating common gamma chain (γ_c)–dependent cytokine signals (Constantinides et al., 2014). While three distinct subsets of helper ILCs (i.e., ILC1, ILC2, and ILC3) and natural killer (NK) cells support of immunity via cytokine release, NK cells additionally show strong cytotoxicity to support anti-microbial and anti-tumor immunity (Diefenbach et al., 2014). Helper ILCs support tissue and immune homeostasis and aid in anti-microbial barrier defense and tissue regeneration or repair (Klose and Artis, 2016). The ILC1 subset and NK cells share surface markers like NK1.1 and NKp46 in C57BL/6 mice, and actively participate in type I immunity (Klose et al., 2014). While sharing functions like IFN-γ production, ILC1 and NK cells differ in their developmental pathways, their central transcriptional networks, and their global tissue distribution in the healthy body (Murphy et al., 2022). ILCs predominantly reside within tissues with minor contribution from circulating precursors, while NK cells are rapidly replaced by newly infiltrating cells from the circulation (Gasteiger et al., 2015).

What are the factors that maintain ILCs within tissues and what are the underlying signals and pathways at play? Previous work demonstrated that γ_c cytokines or transforming growth factor-β (TGF-β) support development or tissue residency in ILCs and NK cells within the salivary gland and tumor microenvironment (TME) (Cortez et al., 2016; Klose et al., 2014). However, whether cooperation of γ_c cytokines and TGF-β is critical for tissue residency of ILCs or NK cells remained unknown.

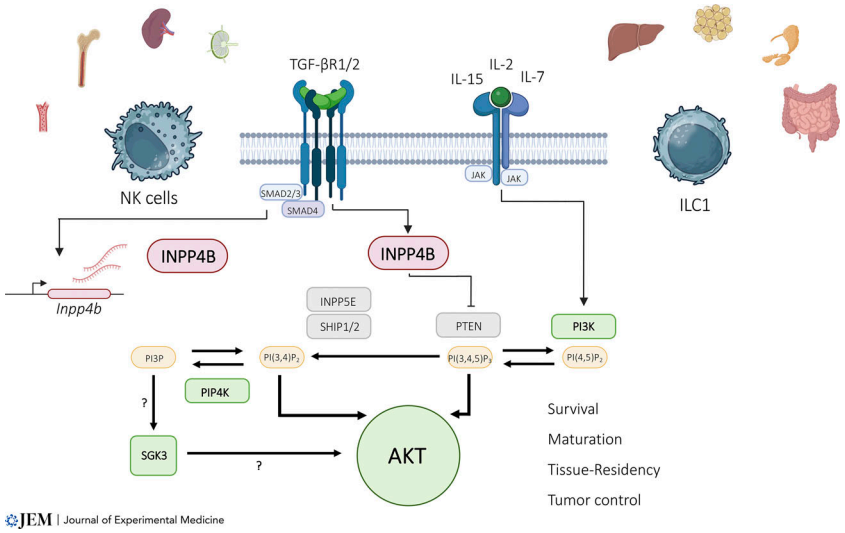
In this issue of *JEM*, Peng et al. (2024) aimed at closing these gaps by examining differential gene expression in bulk RNA sequencing data of ILC1s. Considering the tissue-resident phenotype of ILC1s, the group overlapped their ILC1 signature with the signature of tissue-resident CD8⁺ T cells to compile a set of core genes characteristic of tissue-resident cells. Notably, *Inpp4b*, which encodes for the enzyme inositol polyphosphate 4-phosphatase type II, was markedly enriched in ILC1s. While INPP4B is best known for its paradoxical roles in regulating AKT activity in cancer cells, it also regulates recycling of membrane receptors or AKT-independent activation of cells via the serum/glucocorticoid-regulated kinase family member 3 (SGK3) (Aki et al., 2020; Ferron and Vacher, 2006; Gasser et al., 2014; Hamila et al., 2021). Being highly expressed next to other core ILC1/tissue-resident CD8⁺ T cell genes (e.g., *Zpf683*, *Itga1*, *Icos*, or *Xcl1*), the group next analyzed published single-cell RNA sequencing data to confirm that cells of high *Inpp4b* expression were enriched in tissues and cells of low *Inpp4b* expression were enriched in the spleen and blood. Notably, *Inpp4b* high expression was also observed to be enriched in NK cells within melanoma and breast cancer. The authors' query of gene correlation analysis and gene ontology enrichment pathways revealed that high expression of *Inpp4b* positively correlated with genes associated with cell survival, cell adhesion, leukocyte maturity, and tissue residency (e.g., *Itga1*, *Itgae*, *Bcl2*, and *Cd69*). Conversely, markers highly expressed on circulating lymphocytes, including CD11b, Kif2m, and CD18, negatively correlated with *Inpp4b* expression. High expression of *Inpp4b* was also found to negatively correlated with cytotoxicity (*Gzma* and *Prf*), collectively suggesting that INPP4B acts as a universal marker for tissue-resident ILC1s and CD8⁺ T cells.

Insights from Zi Yan Chen and Arthur Mortha.

To support their initial bioinformatic analyses, Peng et al. (2024) generate *Ncr1*^{iCre}

[1]Department of Immunology, University of Toronto, Toronto, Canada.

Correspondence to Arthur Mortha: arthur.mortha@utoronto.ca.



INPP4B integrates environmental signals via AKT activation to sustain tissue adaptation in ILC1s and NK cells. TGF-β receptor signaling promotes INPP4B expression, allowing the integration of environmental IL-7, IL-15, or IL-2 signaling into the activation of AKT (bold arrows). Control of AKT activation by INPP4B facilitates cells survival and/or maturation, establishment of tissue-residency, and appropriate, local tumor control. The pathways are acting on ILC1s and NK cells in multiple organs during steady state, but also in newly created TMEs. An alternative AKT activation pathway via SGK3 requires validation (indicated by questions marks).

× *Inpp4b*$^{fl/fl}$ mice mediating loss of INPP4B in all NKp46-expressing cells (including ILC1, NK cells, and NCR$^+$ ILC3). Deletion of *Inpp4b* in NCR1$^+$ cells resulted in a significant decrease in liver ILC1s, visceral adipose tissue (VAT) ILC1s, salivary gland (SG) ILC1s, small intestinal (SI) ILC1s, and colonic (CO) ILC1s. Even NK1.1-expressing NCR$^+$ILC3s, known to undergo transition into ILC1s, were affected by the deletion of *Inpp4b*. In contrast, steady-state NK cells in liver, VAT, SG, SI, CO, blood, and spleen, similar to NK1.1$^+$ cells in the lung, were not affected by the deletion, suggesting an important role for INPP4B in regulating tissue residency in ILC1s. Noteworthy, NK cells within lymph nodes and bone marrow displayed alterations in their frequency mirrored by a transient block of NK cell maturation in the bone marrow.

Aiming at resolving the reason for these defects, the authors next compared the gene expression profile of ILC1s sorted from the livers of *Inpp4b*$^{fl/fl}$ and *Ncr1*^{iCre} × *Inpp4b*$^{fl/fl}$ mice. ILC1s from *Inpp4b*-deficient mice displayed an upregulation of pro-apoptotic genes and a downregulation of pro-survival genes and genes regulating cellular metabolism. Peng et al. (2024) did not observe a failure in bromodeoxyuridine incorporation, suggesting that INPP4B participates in regulating the survival of ILC1s in tissues but not their proliferation. NK cells from *Ncr1*^{iCre} × *Inpp4b*$^{fl/fl}$ mice displayed higher levels of cell death, irrespective of the concentration of IL-15. However, given that NK cells were not significantly reduced in all the organs examined in *Inpp4b*-deficient mice, it would be worth exploring whether the NK cells within the tissue were more susceptible to apoptosis, or were more readily repopulated by circulating NK cells. To conduct analysis of signaling pathways in primary cells from *Inpp4b*$^{fl/fl}$ and *Ncr1*^{iCre} × *Inpp4b*$^{fl/fl}$ mice, the authors made use of in vitro expanded NK cells to surpass the obstacle of insufficient quantities of ILC1s these assessments. To their advantage, in vitro cultured NK cells showed an increase in *Inpp4b* expression following stimulation with TGF-β and thus served as a suitable system for the biochemical analysis of signal transduction in the presence or absence of INPP4B. Mimicking the complex tissue environment, the authors explored the phosphorylation of kinases acting downstream of IL-15, IL-2, IL-7, and TGF-β, revealing that *Inpp4b*-deficient NK cells show a profound defect in their ability to phosphorylate AKT in response to these stimuli.

Based on the impact of INPP4B on ILC1 and NK cell survival and cytokine signaling, Peng et al. (2024) decided to investigate the relevance of this biology in the context of malignancies. Intrigued by their in silico analysis revealing that INPP4B high-expressing cell signatures accumulated in tumors, they next examined the effects of *Inpp4b* deficiency on melanoma growth and metastasis. Using either subcutaneously implanted tumors and a metastatic model, the authors revealed a drastic failure of ILC1s and NK cells to thrive in the TME and control tumor growth and metastasis. To determine if these findings were also relevant in breast cancers (characterized by the highest *Inpp4b* signature), Peng et al. (2024) employed an orthotopic model of triple negative breast cancer. While NK cells didn't contribute to the control of these tumors, their observations revealed a general reduction of NK1.1$^+$ cells even in breast cancer, in line with their data in steady state and melanoma. Notably, *Inpp4b* deficiency impacted the differentiation of NK cells in breast cancers, indicating that diverse TMEs, and possibly levels of INPP4B driven by these environments, distinctly affect NK cell subsets and their function, while in general altering ILC1 numbers.

Overall, the results of this study reveal that INPP4B plays defined roles in ILCs by regulating their survival and maturation within the tissue microenvironment. Altering the integration of TGF-β and γ_c cytokine signaling pathways within ILC1s and NK cells drastically affects their ability to establish tissue residency and local control of tumors. However, it remains to be formally shown whether this pathway also impacts CD8$^+$ T cells in their respective states and environments. While this study investigated NK cell numbers within the TME, a transcriptional and metabolic analysis of NK cells and ILC1s within healthy tissues and the TME of control and *Inpp4b* knockout animals could help to clarify how INPP4B aids in dynamically rewiring ILCs to adapt to a changing environment. Such experiments could aid in better understanding the complexity of signals, pathways, and functions that act in driving local anti-tumor immunity. Interrogating the influence of environmental signals in the context of altered INPP4B expression or altered enzymatic activity could provide important insights into additional functions of ILCs (e.g., NK cell memory) or the biology of other innate immune cells. IL-7, IL-2, and IL-15 act upstream of AKT; however, other cytokine and surface receptors on ILCs or tissue-resident myeloid cells may operate through AKT and similarly promote tissue

residency in an INPP4B-dependent fashion. While multiple organs were analyzed in this study, the full extent of *Inpp4b* deficiency, or the nature and regulation of cells promoting INPP4B expression, remains unknown and is an attractive area of new investigation to understand what it takes for immune cells to work within changing environments.

In summary, Peng et al. (2024) present compelling evidence that integrating environmental cytokine signaling through INPP4B plays a critical role in the regulation of tissue residency and break ground for expeditions to explore an aspect of immunology of growing importance.

References

Aki, S., et al. 2020. *Mol. Biol. Cell*. https://doi.org/10.1091/mbc.E19-11-0662

Constantinides, M.G., et al. 2014. *Nature*. https://doi.org/10.1038/nature13047

Cortez, V.S., et al. 2016. *Immunity*. https://doi.org/10.1016/j.immuni.2016.03.007

Diefenbach, A., et al. 2014. *Immunity*. https://doi.org/10.1016/j.immuni.2014.09.005

Ferron, M., and J. Vacher. 2006. *Gene*. https://doi.org/10.1016/j.gene.2006.02.022

Gasser, J.A., et al. 2014. *Mol. Cell*. https://doi.org/10.1016/j.molcel.2014.09.023

Gasteiger, G., et al. 2015. *Science*. https://doi.org/10.1126/science.aac9593

Hamila, S.A., et al. 2021. *Adv. Biol. Regul.* https://doi.org/10.1016/j.jbior.2021.100817

Klose, C.S., and D. Artis. 2016. *Nat. Immunol.* https://doi.org/10.1038/ni.3489

Klose, C.S.N., et al. 2014. *Cell*. https://doi.org/10.1016/j.cell.2014.03.030

Murphy, J.M., et al. 2022. *Front. Immunol.* https://doi.org/10.3389/fimmu.2022.836999

Peng, V., et al. 2024. *J. Exp. Med*. https://doi.org/10.1084/jem.20230124