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INSIGHTS

Tissue-resident NK cells do their own glandscaping

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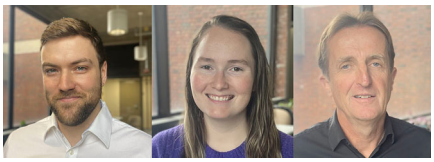
In this issue of *JEM*, Sparano et al. (https://doi.org/10.1084/jem.20240930) present compelling evidence that salivary gland trNK cells originate from cNK cells and are developmentally distinct from ILC1 cells. Mechanistically, they demonstrate that continuous autocrine TGF-β signaling drives salivary gland tissue residency and works in synergy with IL-15 to enhance Hobit-dependent cytotoxicity.

Group 1 innate lymphoid cells (ILCs), comprising natural killer (NK) cells and ILC1s, are essential players in type 1 immune responses against infections and cancer. They exert their effects through direct cell lysis and the production of IFN-γ (Spits et al., 2013). NK cells have historically been cast as a highly cytotoxic circulating population that infiltrates infected tissues to perform their effector functions. ILC1s on the other hand are viewed as a tissue-resident counterpart to NK cells, with reduced cytotoxic potential but enhanced cytokine production (Spits et al., 2013). Advances in single-cell transcriptomics and tissue-specific in vivo genetic tools have demonstrated that these two groups are transcriptionally and ontogenically distinct. However, the lines neatly classifying NK cells as a circulating population, called into tissues only temporarily to control infection, have blurred. Indeed, beginning in the early 2010s, reports emerged of NK cells in mice and humans with ILC1-like traits that establish residency in peripheral tissues during homeostatic (Sojka et al., 2014; Tessmer et al., 2011) and inflammatory conditions (Flommersfeld et al., 2021; Torcellan et al., 2024). Despite these insights, several key questions remain. What roles do tissue-resident NK (trNK) cells play in protecting us from pathogens? What are the developmental origins of trNK cells, and at what point in life do these populations arise? Finally, what signals drive NK cells to establish and maintain tissue residency?

In 2011, our lab discovered a unique population of NK cells within the salivary glands (SGs) of mice that respond to murine cytomegalovirus (MCMV) infection but exhibit a largely hyporesponsive phenotype (Tessmer et al., 2011). Similar to other tissue-resident innate lymphocytes, this subset of cells is characterized as $CD49a^+CD69^+TRAIL^+CD62L^{lo/neg}$, is not thymus-derived, and biases strongly towards host-derived cells in parabiosis experiments (Cortez et al., 2014; Gasteiger et al., 2015). Interestingly, both NFIL3-dependent and -independent NK cells are found in this organ (Cortez et al., 2014; Erick et al., 2016). Like conventional NK cells, SG NK cells require trans-presented IL-15 for survival (Cortez et al., 2014). Given that the SGs are a primary site for CMV replication and latency, it is logical for NK cells, key controllers of CMV infection, to establish a sentinel population in this organ to mitigate viral dissemination. However, mice and humans are unable to completely eradicate CMV from this tissue, reflecting a more nuanced role for these cells. As SG lymphocytes are essential for maintaining oral-pharyngeal immune tolerance to harmless food antigens, SG NK cells likely balance their inflammatory activity with more regulatory functions. Supporting this notion, recent work from our lab demonstrates that SG NK cells restrain cytotoxic $CD8^+$ T cells via the PD-L1/PD-1 axis (Borys et al., 2024). Despite these new understandings, the molecular mechanisms that drive NK cells into this tissue-specific fate have yet to be fully elucidated.



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One critical molecule known to be required for trNK cell formation is TGF-β. In 2016, it was shown that mice with group 1 ILCs deficient in *Tgfbr2*, the receptor for TGF-β, have significantly fewer trNK cells and ILC1. This study demonstrated that TGF-β signaling partially represses Eomes activity, enabling the expression of tissue residency markers such as CD49a, CD69, and CD103 on SG trNK cells (Cortez et al., 2016). Notably, this TGF-β–mediated repression of Eomes parallels the adaptations undergone by tissue-resident memory (T_{RM}) T cells following infection (Mackay et al., 2015). Eomes actively represses *Hobit*, a transcription factor required for the development of tissue-resident lymphocytes. Thus, TGF-β acts as a critical factor in the Hobit-dependent acquisition of tissue-resident features (Parga-Vidal et al., 2021). Despite these similarities between T_{RM} and trNK cell ontogeny, the interplay between these signaling networks during the establishment of tissue residency in NK cells remains poorly understood.

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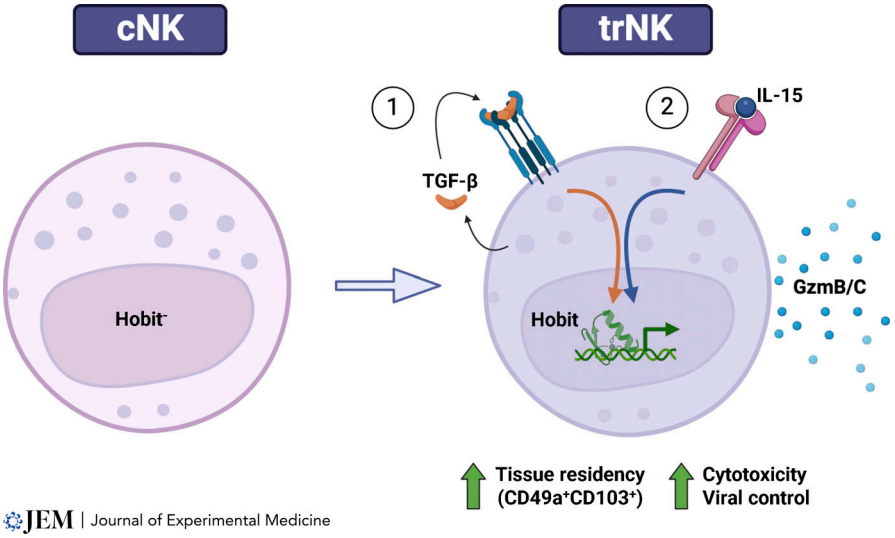

Autocrine TGF-β signaling is required for the transition of conventional NK (cNK) cells to tissue-resident NK (trNK) cells in the salivary glands. IL-15 then synergizes with TGF-β to drive NK cells toward Hobit-dependent cytotoxicity.

In this issue, Sparano et al. shed new light on the mechanisms driving tissue residency in NK cells. The authors began by examining which TGF-β–producing populations might initiate the cascade of events driving NK cells into tissue residency. Notably, they discovered that in addition to expressing *Tgfbr2*, group 1 ILCs themselves abundantly produce TGF-β under homeostatic conditions. To explore the role of group 1 ILC–derived TGF-β, the authors used *NCR1^{Cre}Tgfb1^{fl}* mice in which TGF-β expression is specifically ablated in all group 1 ILCs (Sparano et al., 2024). In these mice, they observed a striking reduction in tissue-resident group 1 ILCs, including trNK cells and ILC1, across multiple glandular tissues such as the SGs, uterus, pancreas, and choroid plexus, while conventional NK cell abundance was increased. Demonstrating the functional relevance of these cells, the authors reported elevated viral titers in the SGs of *NCR1^{Cre}Tgfb1^{fl}* mice following MCMV infection. Importantly, they replicated these findings in an inducible deletion model, suggesting that tissue-resident group 1 ILCs continuously require autocrine TGF-β to retain residency in peripheral tissues. Furthermore, elegant mixed bone marrow chimera experiments ruled out paracrine TGF-β signaling as a mediator of this process, pinpointing autocrine TGF-β as a cell-autonomous driver of trNK cells and ILC1s (Sparano et al., 2024).

Sparano et al. subsequently focused their attention on the temporal dynamics of SG trNK cell ontogeny and function. Group 1 ILCs differ in the manner in which they seed their respective niches, with ILC1s seeding tissues during distinct windows of development while conventional NK cells are continuously replenished from bone marrow precursors (Sparano et al., 2022). However, the ontogeny of trNK cells remains less well-defined. By evaluating neonatal mice, the authors discovered that SG trNK can be detected as early as 1.5 wk of age and expand until adulthood. Through fate-mapping experiments, they showed that the SG trNK pool is seeded by newly generated group 1 ILCs during the neonatal period. Using single-cell RNA sequencing, the authors further explored the functional profiles of SG group 1 ILCs across their developmental spectrum. Using this approach, they uncovered a unique population of NK cells with strong TGF-β pathway activity and elevated levels of granzyme (Gzm) B and C, indicative of cytotoxic potential. Functional validation through ex vivo killing assays revealed that these trNK cells have a more potent cytotoxic ability than conventional SG NK cells (Sparano et al., 2024).

The authors next sought to identify the signals that drive the ontogeny of the TGF-β–induced cytotoxic trNK population. Using Hobit reporter mice and Hobit-deficient mice, they demonstrated that cytotoxic SG GzmB$^+$GzmC$^+$trNK cells rely on Hobit-dependent pathways for development (Sparano et al., 2024). In vitro stimulation assays corroborated these findings, showing that TGF-β induces the expression of tissue residency markers in both mature and immature NK cells. However, treatment with exogenous TGF-β did not result in the emergence of a GzmB$^+$GzmC$^+$ population. The authors ultimately revealed that following TGF-β exposure, treatment with IL-15 partially overcomes this suppression, promoting the emergence of CD49a$^+$GzmB$^+$GzmC$^+$ cells resembling the cytotoxic trNK cells observed in vivo (Sparano et al., 2024). This cytokine combination was found to robustly upregulate the expression of Hobit in NK cells, reinforcing the role of this transcription factor. Taken together, these data suggest that TGF-β, in combination with IL-15, has an imprinting effect on NK cells, pushing them into a Hobit-dependent state of cytotoxicity and tissue residency.

In summary, Sparano et al. uncovered a cell-autonomous role for autocrine TGF-β during the development of group 1 ILCs across multiple glandular organs and further solidified their importance in controlling MCMV infection. Their findings open several promising avenues for future research. First, the broader functional abilities and immunoregulatory roles of trNK cells in the SGs warrant deeper exploration, particularly in light of emerging evidence of novel functions. For example, Schuster et al. identified a population of memory-like SG trNK cells capable of suppressing autoreactive CD4$^+$ T cells during chronic MCMV infection (Schuster et al., 2023). Recent work from our lab suggests that SG NK cells may provide peripheral tolerance against autoimmune CD8$^+$ T cells (Borys et al., 2024). Second, a role for the microbiome in regulating the immune landscape of the SGs has been proposed (Zubeidat et al., 2023), making it highly relevant to investigate how commensal microbes influence trNK cell development and function. Last, the trNK populations across the three SGs—submandibular, sublingual, and parotid—remain incompletely characterized (Zubeidat et al., 2023). While trNK cells are abundant in the submandibular glands, they constitute a smaller fraction of lymphocytes in the sublingual and parotid glands, and their specific immune

roles in these organs are not yet fully understood.

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