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INSIGHTS

Innatus immunis: Evolving paradigm of adaptive NK cells

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The mechanisms that govern the development of adaptive-like NK cells are elusive. Shemesh et al. (2022. *J. Exp. Med.* https://doi.org/10.1084/jem.20220551) report that the development of FcRγ$^{-/low}$ adaptive-like NK cells requires reduced mTOR activity and depends on TGF-β or IFN-α. These findings provide exciting new molecular blueprints explaining the development and functions of adaptive-like NK cells.

NK cells constitute the largest lymphocyte subset of the innate immune system and recognize viral proteins or induced-self proteins on infected and malignant cells. NK cells do not express clonotypic receptors but rely on germline-encoded conserved activation receptors. Irrespective of their inherent, innate qualities, a subset of NK cells can develop an adaptive-like phenotype (see Fig. 1) and go on to form a reservoir of short-term memory cells.

The formation of adaptive-like natural killer (NK) cells is well-established (Kamimura and Lanier, 2015). Infections with CMV, influenza, vesicular stomatitis virus, and HIV-1 led to the formation of adaptive-like NK cells (Paust et al., 2010; Sun et al., 2009). These adaptive-like NK cells expanded following secondary infections with vaccinia and HSV-2. Human CMV (HCMV) or SARS-CoV-2 infections led to the accumulation of adaptive-like CD52^{+}NKG2C^{+}CD94^{+} NK subset and correlated with the latent or COVID-19 pathology, respectively (Chung Guo et al., 2022). Recognition of unique peptide/MHC Class I complexes by the activating NKG2C or the inhibitory NKG2A is reminiscent of classical CD8^{+} T cell responses. Presentation of UL40-derived VMAPRTLIL, presented by HLA-E and recognized by the NKG2C/CD94 complex, is a prerequisite for generating adaptive-like NK cells during HCMV infection.

In recent years, the phenotypic characteristics of adaptive-like NK cells have been well-established. However, the transcriptional mechanism governing the development of adaptive NK cells is yet to be fully determined. In this issue of *JEM*, Shemesh et al. (2022) establish a link between the lower expression of FcRγ and the development of adaptive-like NK cells. FcRγ contains two immunoreceptor tyrosine-based activation motifs (ITAMs). It forms homodimeric (γ-γ) or heterodimeric complexes with CD3ζ, which comprises three ITAMs (Medjouel Khlifi et al., 2022). The FcRγ associates with Type-I family members, including FcεRI, FcαRI, FcγRI, and FcγRIIIA. FcRγ also transduces exogenous signals of transmembrane receptors such as Dectin-1&2, LMIR8, MARR-I&II, OSCAR, TREM, PIR-A, active γδTCR, IL-3R, and NKp46 (Brandsma et al., 2016). Indeed, the lack of FcRγ resulted in the complete absence of NKp46 (NCR1), and a higher expression of CD3ζ alone failed to rescue this defect (Duhan et al., 2019). Given this background, how does a lower expression of FcRγ help in the establishment of adaptive features in mature NK cells? Why is a reduction in an essential adaptor protein required to form memory-like NK cells?

Insights from Mohamed Khalil and Subramaniam Malarkannan.

This current study from Lanier's lab provides extensive mechanistic insights into how the lower expression of FcRγ may regulate the formation of adaptive NK cells. Exposure to rapamycin, a reduction in f mammalian target of rapamycin complex-1 (mTORC1) or mTORC2 functions, and activation by TGF-β or IFN-α resulted in the lower expression of FcRγ. These findings provide valuable information and add to our understanding; however, they raise new questions. One of the major functional roles of mTORC1 is to promote protein synthesis and cell proliferation in response to cytokine stimuli. The causal relationship established in the current study using NK cells from COVID-19 or rapamycin-treated lung transplant patients implies that mTORC1

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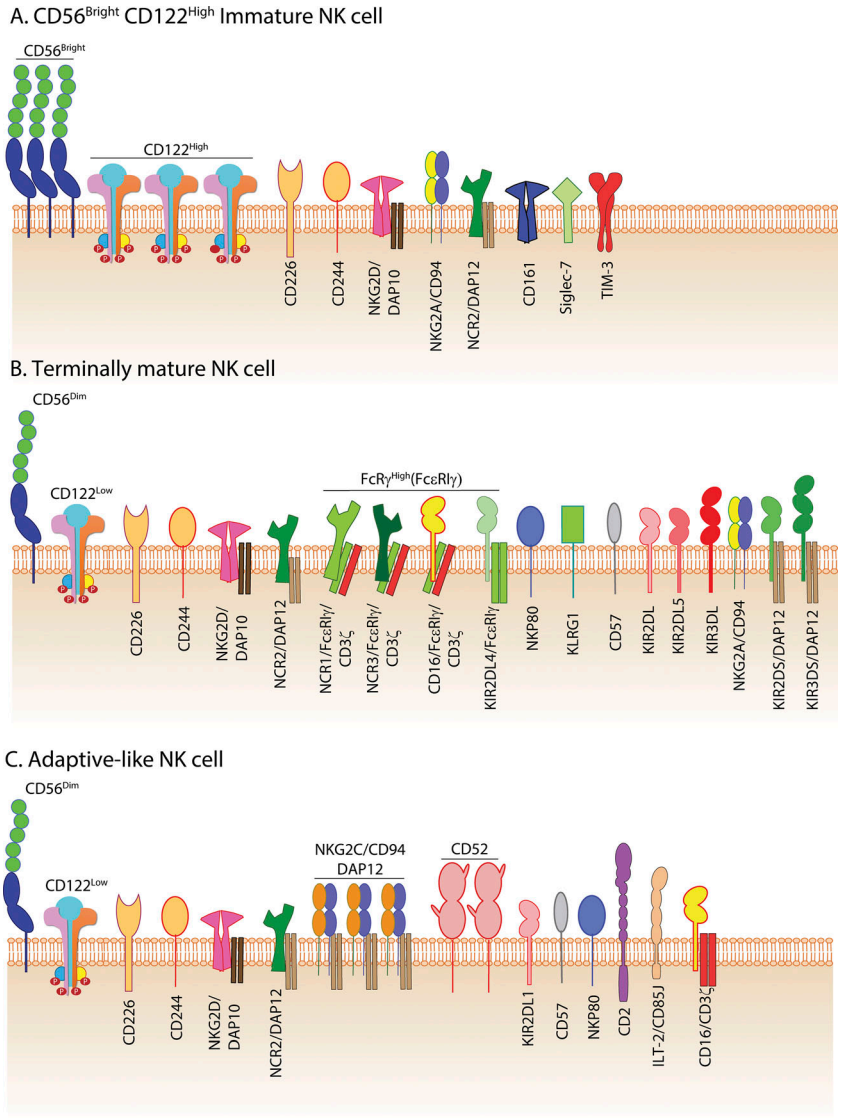

Figure 1. Phenotype of adaptive-like NK cells differs from iNK or mNK cells. (A–C) Major phenotypic characteristics of CD56^{Bright}CD122^{High} immature NK (A), CD56^{Dim}CD122^{Low} terminally mature NK (B), and adaptive-like NK cells (C). Adaptive-NK cells are defined by a higher cell surface expression of NKG2C/CD94/DAP12 complexes and a lower expression of FcRγ. Since FcRγ functions as an essential adaptor protein for several activating receptors, including NCR1, their expression is limited in adaptive-like NK cells. This is an active research area, and therefore additional biomarkers are expected.

controls the expression of FcRγ. However, is this control exerted at the transcriptional level or through an m^{7}G-Cap–dependent translation of *FCER1G* mRNA? Earlier studies have shown that the terminal maturation of NK cells requires Tsc1-dependent negative regulation of IL-15–triggered mTORC1 activation (Yang et al., 2016). If true, do adaptive NK cells need additional regulatory elements apart from mTORC1 that differ from maturing NK cells? Do adaptive NK cells differentiate from a subset of terminally mature NK cells? Or do they originate from a distinct population of immature NK cells where TGF-β or IFN-α can limit the activation and functions of mTORC1?

The current study implicates that TGF-β and IFN-α suppressing mTOR pathways is controversial and potentially context-dependent. Type-I IFNs are essential in forming adaptive-like NK cells in elderly COVID-19 patients (Guo et al., 2022). Signals via IFNAR lead to STAT1 and STAT4 activation resulting in the blockade of cell proliferation and phosphorylation of Ser-ine^{473} of Akt by mTORC2 (Kaur et al., 2012). Treatment of mouse or human NK cells with TGF-β in vitro blocked IL-15–induced mTOR activation, which concurs with the current study (Viel et al., 2016). However, TGF-β differentiates immature CD56^{Bright}CD94$^+$NKp80$^+$CD16$^-$CD57$^-$ NK cells into intraepithelial-ILC1-like NK cells with augmented effector functions (Moreno-Nieves et al., 2021). These studies emphasize that additional work is needed to determine the context under which TGF-β and IFN-α help generate adaptive-like NK cells. A link between the diminished proliferation and reduced expression of FcRγ forms the basis for developing adaptive-like NK cells. However, the cell-intrinsic and cell-extrinsic functional roles of FcRγ are yet to be fully appreciated. For example, NCR1 requires FcRγ for its signal transduction. The absence of FcRγ leads to a significant reduction in NCR1 expression, resulting in augmented anti-viral CD8$^+$ T cell responses (Duhan et al., 2019). Thus, not only do the adaptive-like NK cells lack the ability to kill and suppress CD8$^+$ T cells, but they also help facilitate an ongoing T cell response.

Another interesting question this paper raises is from which stage of development do adaptive-like NK cells originate? The functional status and the interplay between mTORC1 and mTORC2 may answer this question. In mouse NK cells, mTORC1 upregulates the expression of Eomesodermin (EOMES) and the transition from CD27$^+$ to CD27$^+$CD11b$^+$. In contrast, mTORC2 facilitates the terminal CD11b$^+$ NK cell maturation through the mTORC2–Akt^{S473}–FoxO1 axis (Yang et al., 2020). In addition, mTORC2 upregulates the expression of T-bet. Eomes and T-bet are members of the T-box family that contain highly conserved DNA-binding domains with differing interacting partners. FoxO1 has a forkhead and winged-helix DNA-binding domains. It is expressed significantly higher in precursors (NKPs) and immature NK (iNK) cells than in terminally mature NK (mNK) cells (see Fig. 2).

Furthermore, the lack of Rictor, an essential component of mTORC2, augmented the expression of FoxO1. Thus, data using human NK cells by Shemesh et al. (2022) validate earlier observations that FoxO1 suppresses the transition of murine iNK to mNK cells through the axis of mTOR2–Akt^{S473}–FoxO1–T-bet (Yang et al., 2018). iNK cells express high levels of FoxO1, which may serve as a checkpoint to preserve the orderly gene expression and allow for proper developmental processes by

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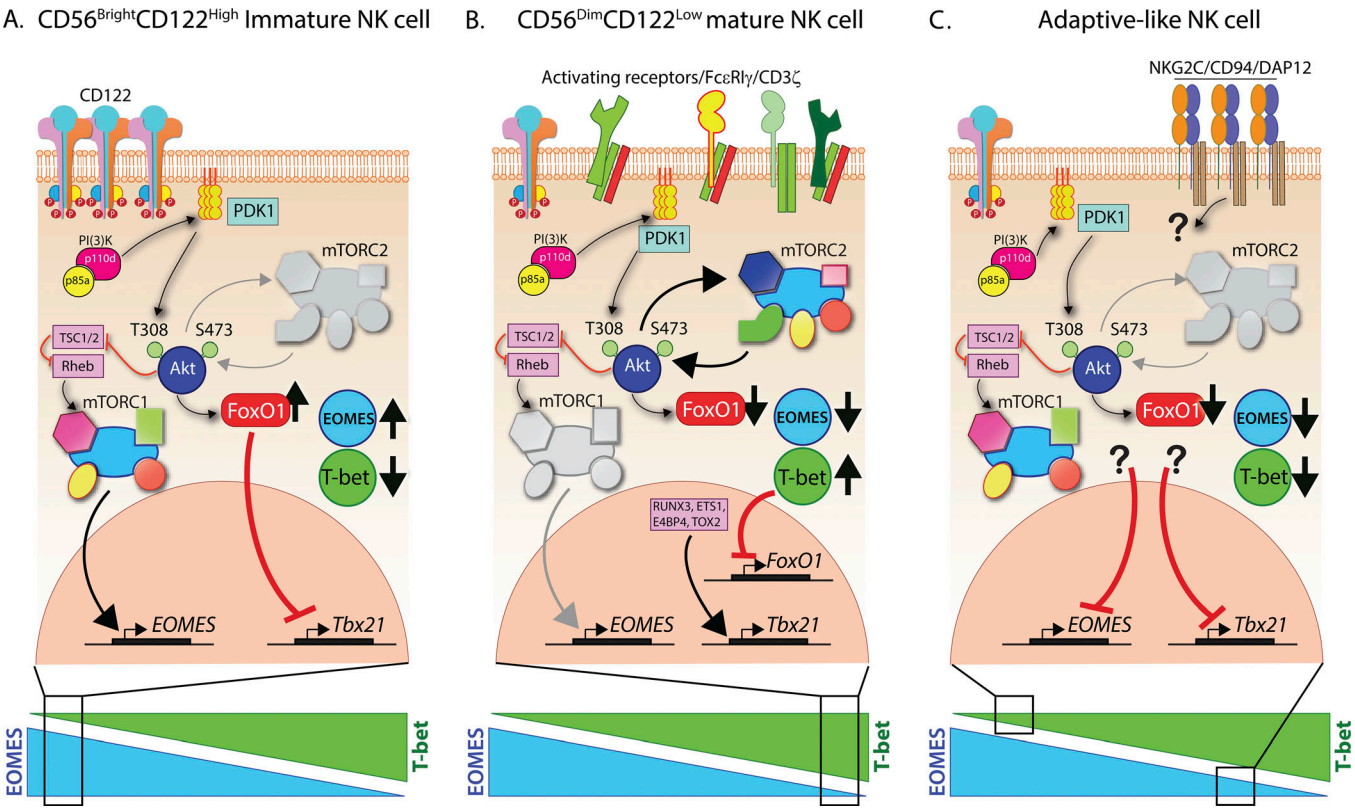

Figure 2. Potential roles of mTORC1 and mTORC2 in programming the generation of adaptive-like NK cells. (A) CD56^{Bright}CD122^{High} iNK cells possess a higher EOMES essential for turning on multiple transcriptional pathways required for orderly development. mTORC1 is required for the expression of EOMES. (B) CD56^{Dim}CD122^{Low} terminally mNK cells require T-bet to transcribe genes involved in cytotoxicity and inflammatory cytokine/chemokine production. (C) The current study describes adaptive-like NK cells possess a lower expression of FoxO1, EOMES, and T-bet. The downstream signaling of NKG2C/CD94/DAP12 complex (or other receptors) that regulates the transcriptional repression of FoxO1, EOMES, or Tbx21 is unknown. Additionally, what alternate transcription factors and regulons are required to generate and maintain adaptive-like NK cells are yet to be determined (question marks).

suppressing several master transcription factors, including T-bet (see Fig. 2). Indeed, FoxO1, with the help of Sp1, binds to the proximal promoter of *Tbx21*, the gene encoding T-bet (Deng et al., 2015). Thus, the classification of the human iNK cells as FcRγ^{-}CD122^{Hi}mTORC2^{Low}-FoxO1^{Hi}EOMES^{Hi}T-bet^{Low} follows previously established observations.

How do adaptive-like NK cells differ from iNK cells? For example, do adaptive-like NK cells develop only after a viral infection or malignant transformation? Or are they a subset of NKPs ready to transition following a pathological insult? Answers to these two questions are essential to determine their developmental trajectory and clinical application. Shemesh et al. (2022) present evidence that adaptive-like NK cells possess the phenotype of FcRγ$^{-/Low}$CD122^{Low}mTORC2^{Low}-FoxO1^{Low}EOMES^{Low}T-bet^{Low}. Unexpectedly, adaptive-like NK cells do not express a higher level of T-bet, an essential requirement for optimal effector functions (see Fig. 2). A lower expression of CD122 demonstrates that these cells are weaned away from IL-2 or IL-15, thereby retaining only a limited proliferative capacity. It is likely that iNK cells, when exposed to specific environmental conditions, including but not limited to TGF-β and IFN-α, may proceed to develop into and express adaptive-like features.

How do terminally mNK cells differ from the adaptive-like NK cells? In contrast to immature or adaptive-like cells, mNK cells require T-bet to turn on genes governing cytotoxicity and inflammatory cytokines or chemokines. A coordinated network of several transcription factors, including Runx3, ETS1, E4bp4, Ets-1, and Tox2 are essential to initiate and sustain the continued expression of T-bet. While these transcription factors are necessary for the transition of iNK to mNK cells, their role in developing into adaptive-like NK cells is yet to be determined. Furthermore, a reciprocal suppression mediated by increasing levels of murine T-bet reduces the levels of both FoxO1 and EOMES in mNK cells, differentiating them from adaptive-like NK cells. mTORC2 minimizes the quantity of cytoplasmic FoxO1 by phosphorylating and directing it for proteasomal degradation. Rictor-deficient and T-bet–deficient murine NK cells cannot produce optimal levels of IFN-γ or mediate in vivo anti-tumor cytotoxicity, underscoring the need for the mTORC2–T-bet axis for effector functions.

In this context, Shemesh et al. (2022) define the terminally mature human NK cells as FcRγ^{Hi}CD122^{Low}mTORC2^{Hi}-FoxO1^{Low}EOMES^{Low}T-bet^{Hi}, which is consistent with earlier findings. However, in the absence of optimal T-bet levels, how adaptive-like NK cells mediate their effector functions is unknown. In the current study, a reduced mTORC2 did not result in an augmented expression of FoxO1, indicating that additional molecular mechanisms are yet to be unraveled. Are the adaptive-like NK cells exhausted or can they become

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exhausted? A reduction in T-bet levels is reminiscent of exhausted $CD8^+$ T cells during chronic viral infections. What are the biological advantages if adaptive-like NK cells are indeed exhausted? It is more likely that the adaptive-like NK cells are not exhausted. What is the immunological advantage of downregulating NCR1 that solely appears to use the FcRγ (four ITAMs)? Earlier findings have shown absence of FcRγ increased the response through CD16 (Liu et al., 2020). Does this mean that the advantage of reducing the expression levels of FcRγ is to augment antibody-dependent cell cytotoxicity of virus-infected cells? Is this achieved by exclusively using the homodimeric CD3ζ (six ITAMs) instead of homodimeric FcRγ (four ITAMs) or heterodimeric FcRγ/CD3ζ (five ITAMs)? If true, what are the quantitative and qualitative differences in the downstream signaling pathways? Given the significant emphasis on the clinical relevance of adaptive-like NK cells, it is imperative to answer these questions.

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