

# Striking a balance: the Goldilocks effect of CD8 $\alpha$ expression on NK cells

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**NK cells are cytotoxic innate immune cells involved in antitumor immunity, and they provide a treatment option for patients with acute myeloid leukemia (AML). In this issue of the *JCI*, Cubitt et al. investigated the role of CD8 $\alpha$ , a coreceptor present on approximately 40% of human NK cells. IL-15 stimulation of CD8 $\alpha$ <sup>-</sup> NK cells induced CD8 $\alpha$  expression via the RUNX3 transcription factor, driving formation of a unique induced CD8 $\alpha$  (iCD8 $\alpha$ <sup>+</sup>) population. iCD8 $\alpha$ <sup>+</sup> NK cells displayed higher proliferation, metabolic activity, and antitumor cytotoxic function compared with preexisting CD8 $\alpha$ <sup>+</sup> and CD8 $\alpha$ <sup>-</sup> subsets. Therefore, CD8 $\alpha$  expression can be used to define a potential dynamic spectrum of NK cell expansion and function. Because these cells exhibit enhanced tumor control, they may be used to improve in NK cell therapies for patients with AML.**

## Regulation of NK cell activation

NK cells are specialized innate lymphoid cells that mediate cellular cytotoxicity without the need for antigen priming (1). Their functional responses are regulated by a balance between signals from activating and inhibitory receptors (2–5). The killer cell immunoglobulin-like receptor (KIR) family encodes both inhibitory and activating members that recognize HLA class I (6). In healthy conditions, inhibitory signals predominate. However, downregulation of HLA molecules on virally infected or malignant cells (7) releases inhibition, tipping the balance toward activation. NKG2A, another inhibitory receptor, binds to HLA-E molecules that are frequently upregulated on the surface of tumor cells to protect them from NK cell-mediated killing (6). NK cells express multiple activating receptors, including natural cytotoxicity receptors and NKG2D, which recognize stress-induced ligands. NK cells also express CD16,

which triggers lysis of antibody-coated target cells via antibody-dependent cell-mediated cytotoxicity.

## CD8 $\alpha$ expression on NK cells and antitumor immunity

The effect of CD8 $\alpha$  on NK cell function is context dependent. CD8 $\alpha$ <sup>+</sup> NK cells exhibit greater cytotoxic function against leukemia cells (8, 9). However, prior work by Fehniger and colleagues using cytokine-induced memory-like NK cells demonstrated that high levels of CD8 $\alpha$  on donor memory-like NK cells correlated with treatment failure in patients with relapsed/refractory acute myeloid leukemia (AML) after adoptive transfer (10). In this issue of the *JCI*, Cubitt et al. (11) focused their investigation on conventional healthy NK cells, subtyped as immunomodulatory CD56<sup>bright</sup> and cytotoxic CD56<sup>dim</sup>, to elucidate the fundamental biological role of CD8 $\alpha$  on NK cells. Both NK cell populations expressed CD8 $\alpha$ , though

levels were higher on CD56<sup>dim</sup> NK cells. The CD8 $\alpha$  homodimer was the predominant (95%) receptor complex expressed. Single-cell RNA sequencing comparing CD8 $\alpha$ <sup>-</sup> and CD8 $\alpha$ <sup>+</sup> NK cells demonstrated no differences in transcript expression profiles, indicating that CD8 $\alpha$  expression does not define a distinct NK cell population in the maturation sequence. To evaluate the effect of CD8 $\alpha$  on tumor control, the authors administered CD8 $\alpha$ <sup>+</sup> and CD8 $\alpha$ <sup>-</sup> CD56<sup>dim</sup> NK cells in a xenogenic K562 leukemia model and observed lower tumor burden in the CD8 $\alpha$ <sup>-</sup> NK cell treatment cohort. These data, supported by additional in vitro experiments, suggest that CD8 $\alpha$  dampens the cytotoxic function of NK cells (11).

## CD8 $\alpha$ and IL-15 signaling

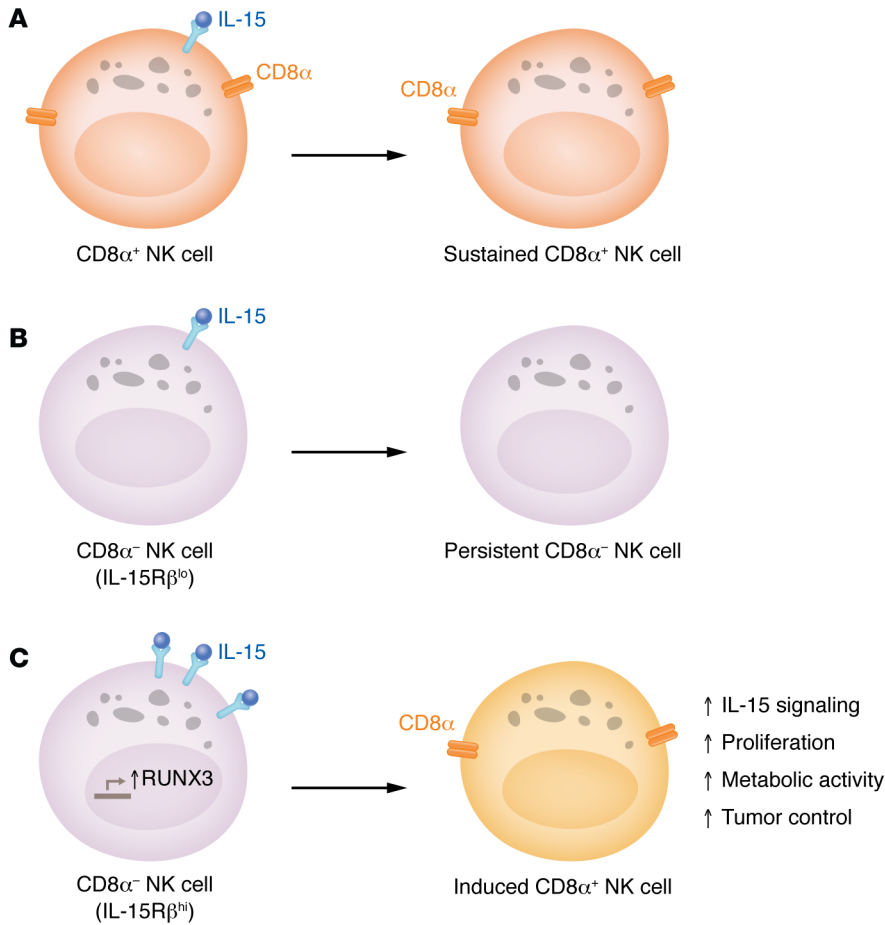
NK cells are dependent on IL-15 for survival and proliferation. IL-15 also primes NK cells and enhances their cytotoxic function against cancer cells (12). Having evaluated the effect of CD8 $\alpha$  on cytotoxicity, Cubitt and colleagues then sought to study the potential role of CD8 $\alpha$  in IL-15 signaling and proliferation (11). They found that CD8 $\alpha$ <sup>-</sup> NK cells had greater survival and proliferation in response to IL-15 in vitro. In line with these findings, CD8 $\alpha$ <sup>-</sup> NK cells expanded more compared with their CD8 $\alpha$ <sup>+</sup> counterparts in xenogeneic adoptive transfer experiments with IL-15 dosing. The authors also asked whether IL-15 controls CD8 $\alpha$  expression and found that IL-15 induced a subset of CD8<sup>+</sup>CD56<sup>dim</sup> cells to upregulate CD8 $\alpha$ , constituting an induced CD8 $\alpha$ <sup>+</sup>CD56<sup>dim</sup> (iCD8 $\alpha$ <sup>+</sup>) population (Figure 1). This phenomenon seemed more pronounced in CD56<sup>bright</sup> cells, though the authors mainly focused on CD56<sup>dim</sup> NK cells. Interestingly, only CD8 $\alpha$  expression increased, not CD8 $\alpha$  $\beta$ , suggesting that IL-15 specifically regulates CD8 $\alpha$ . Further analysis of this population revealed that iCD8 $\alpha$ <sup>+</sup>CD56<sup>dim</sup> cells exhibited more proliferation in response to IL-15 in vitro and in vivo compared with CD8 $\alpha$ <sup>-</sup>

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**Figure 1. Activation by IL-15 generates induced CD8 $\alpha^+$  NK cells.** Cubitt et al. (11) present three scenarios for human NK cells responding to IL-15. **(A)** In the first scenario, CD8 $\alpha^+$  NK cells receive an IL-15 signal and become sustained CD8 $\alpha^+$  NK cells. **(B)** In the second scenario, CD8 $\alpha^-$  NK cells with low expression of IL-15R $\beta$  are activated by IL-15 and fail to upregulate CD8 $\alpha$ , becoming persistent CD8 $\alpha^-$  NK cells. **(C)** In the third scenario, CD8 $\alpha^-$  NK cells with high expression of IL-15R $\beta$  upregulate RUNX3 upon IL-15 stimulation and become induced CD8 $\alpha^+$  NK cells. These cells exhibit several beneficial properties, including enhanced tumor control.

(termed persistent) and original CD8 $\alpha^+$  (termed sustained) counterparts (Figure 1). Mechanistically, RUNX3, a transcription factor that has predicted binding sites in the *CD8A* locus, showed potential for interaction. CRISPR-mediated deletion of RUNX3 abolished the ability of IL-15 to induce CD8 $\alpha$  expression and resulted in decreased expression levels of CD8 $\alpha$  in sustained CD8 $\alpha^+$  NK cells.

To delineate the mechanism upstream of RUNX3, Cubitt and authors investigated components of the IL-15 receptor: IL-15R $\alpha$  (also known as CD25), IL-2/15R $\beta$  (also known as CD122), and common  $\gamma$  chain (also known as CD132) and activation of downstream effectors. iCD8 $\alpha^+$ CD56 $^{dim}$  NK cells had higher expression of CD132 and IL-2/15R $\beta$  and downstream

phospho-activated ERK, STAT5, AKT and S6. Furthermore, iCD8 $\alpha^+$ CD56 $^{dim}$  NK cells originated from CD8 $\alpha^-$ CD56 $^{dim}$  NK cells that had higher preexisting CD122 expression. Induction of CD8 $\alpha$  appears to occur downstream of IL-15 signaling, but CD8 $\alpha$  itself does not seem to drive proliferation or survival. This premise was supported by the observation that CD8 $\alpha$  KO did not augment NK cell expansion or survival with IL-15. Given that IL-15 signaling is known to regulate metabolism in NK cells(11), the authors examined the metabolic profile of iCD8 $\alpha^+$  NK cells. iCD8 $\alpha^+$ CD56 $^{dim}$  NK cells exhibited higher expression of nutrient transport proteins and increased glucose uptake compared with persistent CD8 $\alpha^-$  and sustained CD8 $\alpha^+$ CD56 $^{dim}$  NK cells. Glycolytic capacity correlated pos-

itively with CD8 $\alpha$  induction, suggesting that greater nutrient uptake and metabolic capacity supports the enhanced IL-15-dependent proliferative capacity of iCD8 $\alpha^+$  NK cells.

### iCD8 $\alpha^+$ NK cells in tumor control

Cubitt and authors evaluated the antitumor function of iCD8 $\alpha^+$  NK cells using a xenogeneic adoptive transfer model with K562 tumor cells (11). After injecting CD8 $\alpha^+$  and CD8 $\alpha^-$ CD56 $^{dim}$  NK cells and monitoring tumor kinetics for 19 days, they isolated 3 NK cell populations: sustained CD8 $\alpha^+$ , persistent CD8 $\alpha^-$ , and iCD8 $\alpha^+$  NK cells. Ex vivo, iCD8 $\alpha^+$  NK cells remained most responsive to IL-15 and maintained robust cytolytic activity against K562 when rechallenged. To determine whether CD8 $\alpha$  directly affected NK cell activation, the authors examined activating receptor signaling in CD8A-KO CD56 $^{dim}$  NK cells. CD8A KO had minimal effect on lysis of K562 or HL60 cells. Evaluation of receptor signaling showed greater degranulation in CD8A-KO cells specifically after NKp30 stimulation. Surprisingly, further investigation did not reveal differences in phospho-activation of signaling molecules downstream of NKp30 engagement. Accordingly, the authors hypothesized that CD8 $\alpha$  may augment inhibitory KIR signaling, because KIR and CD8 $\alpha$  both bind to HLA molecules. Indeed, calcium flux assays with NKp30 and inhibitory KIR stimulation demonstrated increased NK cell activation in with loss of CD8 $\alpha$ . The authors conclude that CD8 $\alpha$  suppresses NKp30 NK cell activation by potentiating inhibitory KIR signaling, though it is likely that additional mechanisms are at play.

### Therapeutic implications and future directions

The findings presented in Cubitt et al. provide insights into the dynamic reprogramming of NK cells and raise possibilities for advancing NK cell therapies (11). IL-15 priming increases NK cell cytolytic function (12). However, prolonged IL-15 stimulation results in NK cell exhaustion, characterized by decreased tumor control and diminished mitochondrial metabolic function (13). In this context, it will be useful to determine the trajectory of CD8 $\alpha$  expression in relation to NK cell exhaus-

tion. Do NK cells begin as CD8 $\alpha$ , acquire CD8 $\alpha$  upon IL-15 exposure to become the iCD8 $\alpha$ <sup>+</sup> as described by Cubitt et al., and then ultimately become exhausted, known in this context as “sustained” CD8 $\alpha$ <sup>+</sup>? The enhanced metabolic activity of iCD8 $\alpha$ <sup>+</sup> NK cells compared with sustained CD8 $\alpha$ <sup>+</sup> NK cells suggests this possibility, though detailed studies are required. Furthermore, while Cubitt et al. (11) mainly focused on CD56<sup>dim</sup> NK cells, the magnitude of CD8 $\alpha$  induction was greatest in CD8 $\alpha$ -CD56<sup>bright</sup> cells. It will be helpful to further characterize iCD8 $\alpha$ <sup>+</sup>CD56<sup>bright</sup> populations and their effect on tumor control, as IL-15-primed CD56<sup>bright</sup> NK cells can exhibit robust anti-tumor cytolytic activity (14).

The results of the study by Cubitt et al. (11) have important therapeutic implications. Perhaps CD8 $\alpha$ -CD56<sup>dim</sup> or iCD8 $\alpha$ <sup>+</sup>CD56<sup>dim</sup> are favorable for adoptive NK cell therapy in cancer, though additional tumor models need to be tested to prove preclinical efficacy. iCD8 $\alpha$ <sup>+</sup> NK cells displayed a potential memory-like phenotype, as they were able to kill tumor cells when rechallenged. How do iCD8 $\alpha$ <sup>+</sup> NK cells directly compare with CD8 $\alpha$ <sup>+</sup> cytokine-induced memory-like NK cells (15, 16)? Finally, from a mechanistic perspective, further investigation into the role

of CD8 $\alpha$  is needed to clearly define its activating or inhibitory function on NK cell cytotoxicity. Furthermore, the role of CD8 $\alpha$  in antibody-dependent cell-mediated cytotoxicity has not been explored in detail. Yet, for now, the main value of CD8 $\alpha$  in NK cells may be the fact that they can define a highly responsive anti-tumor population that may be exploited to improve NK cell therapies for patients.

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