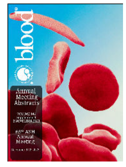
Blood 142 (2023) 3925–3926



The 65th ASH Annual Meeting Abstracts

POSTER ABSTRACTS

203.LYMPHOCYTES AND ACQUIRED OR CONGENITAL IMMUNODEFICIENCY DISORDERS

The NK Cell Receptor NKp46 Acts As an Essential Regulator of ILC1 Proliferation, Effector Function and Anti-AML Activity

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Innate lymphoid cells (ILCs) are tissue-resident innate immune cells that respond rapidly to infection and secrete inflammatory mediators. Group 1 includes ILC1s and NK cells. Both cell types produce IFN-γ and require T-BET for their function; however, at least in mice, ILC1s do not express EOMES while NK cells do. The NKp46 receptor, encoded by the *Ncr1* gene, plays an important role in NK cells. Non-NK immune cells, including ILC1s also express NKp46. However, the role of NKp46 in these non-NK immune cells remains largely unknown. We previously reported that NKp46 deficiency reduces both the percentages and numbers of ILC1s in mice but not the proportions of NK cells, ILC2s, and ILC3s (Wang *et al.*, *Plos Biology*, 2018), suggesting that NKp46 may play a unique role in ILC1s. However, the precise mechanism by which NKp46 regulates ILC1s is yet to be determined.

Using RNA-seq, we identified 35 upregulated genes and 66 downregulated genes in mutant ILC1s (*Ncr1* $^{gfp/gfp}$) compared to wild-type (*Ncr1* $^{+/+}$)ILC1s ($p < 0.05$). Among the genes that were differentially expressed, we noted decreased expression of the cytokine receptors *Il-18r1*, *Il-7rα*, and *Il-2rα* in *Ncr1* $^{gfp/gfp}$ILC1s compared to *Ncr1* $^{+/+}$ILC1s. Using gene set enrichment analysis of RNA-seq data from freshly sorted ILC1s from the livers of *Ncr1* $^{+/+}$and *Ncr1* $^{gfp/gfp}$mice, we identified the top 10 pathways associated with downregulated genes. Notably, NKp46 deletion significantly affected NF-κB. We validated this finding by treating ILC1s with NKp46 antibody and found that the phosphorylation of NF-κB p65 subunit was increased.

The downregulation of IL-2Rα and IL-7Rα on *Ncr1* $^{gfp/gfp}$ILC1s led us to investigate whether NKp46 also regulates ILC1 proliferation, survival, or persistence. We observed comparable levels of apoptosis in ILC1s freshly isolated from *Ncr1* $^{+/+}$and *Ncr1* $^{gfp/gfp}$mice. Thus, lack of NKp46 does not affect ILC1 apoptosis. However, expression of the proliferation marker Ki67 was significantly downregulated in freshly isolated *Ncr1* $^{gfp/gfp}$ILC1s compared to *Ncr1* $^{+/+}$ILC1s. We also intravenously (*i.v.*) co-transferred equal numbers of FACS-sorted *Ncr1* $^{+/+}$ and *Ncr1* $^{gfp/gfp}$ILC1s labeled with CellTrace^{TM} Violet dye (to distinguish donor cells from host cells) into immunodeficient recipient *Rag2* $^{-/-}$ *Il2γc* $^{-/-}$ mice. After one week, the population of *Ncr1* $^{gfp/gfp}$ILC1s was less than those of *Ncr1* $^{+/+}$ILC1s in the recipient *Rag2* $^{-/-}$ *Il2γc* $^{-/-}$ mice ($p = 0.0002$, n = 5), indicating that ILC1 proliferation requires NKp46 in vivo.

To investigate the functional relevance ofNKp46, we *i.v.* injected C1498 AML cells into *Ncr1* $^{+/+}$or *Ncr1* $^{gfp/gfp}$mice. The *Ncr1* $^{gfp/gfp}$mice exhibited significantly more tumor growth than the *Ncr1* $^{+/+}$mice. Animal survival was significantly shorter in the *Ncr1* $^{gfp/gfp}$mice compared to *Ncr1* $^{+/+}$mice ($p = 0.0023$, n > 9). Injection of additional *Ncr1* $^{+/+}$ILC1s into *Ncr1* $^{+/+}$mice increased survival compared to animals without additional *Ncr1* $^{+/+}$ILC1 administration ($p = 0.0183$, n = 6). Further, this injection managed to rescue the dramatic decrease in the number of ILC1s observed in the *Ncr1* $^{gfp/gfp}$mice. This increased survival was in stark contrast to the *Ncr1* $^{gfp/gfp}$mice without *Ncr1* $^{+/+}$ILC1 injection, or those who received *Ncr1* $^{gfp/gfp}$ILC1 injection.

We also investigated the role of NKp46 in human ILC1s. Human ILC1s isolated from peripheral blood mononuclear cells (PBMCs) were treated with a human NKp46 antibody, increasing Ki67 expression. Next, we sorted NKp46 $^{-}$ and NKp46 $^{+}$

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ILC1s from human PBMCs and co-cultured them with MOLM13 AML cells for 24 h and found the human NKp46 $^{-}$ ILC1s were significantly less cytotoxic compared to NKp46 $^{+}$ ILC1s.
In conclusion, NKp46 is indispensable for ILC1 expression of IL-2Rα. Deficiency of NKp46 in mice reduces IL-2Rα expression on ILC1s by downregulating NF-κB signaling, thus impairing ILC1 proliferation. In a mouse model of AML, we see the functional consequences of cellular impairment as NKp46 deficiency eliminates the ability to control tumor growth and reduces survival. These changes can be reversed by injection of sufficient numbers of NKp46 $^{+}$ ILC1s into mice deficient for the receptor. Human NKp46 $^{+}$ ILC1s also exhibit stronger cytokine production and cytotoxicity than their NKp46 $^{-}$counterparts, suggesting that NKp46 plays a similar role in both humans and mice. Our findings suggest a novel strategy for activating NKp46 signaling to promote ILC1 proliferation and anti-tumor activity.

Disclosures No relevant conflicts of interest to declare.

https://doi.org/10.1182/blood-2023-191070

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