DIFFERENTIAL EFFECTS ON NATURAL KILLER CELL PRODUCTION BY MEMBRANE-BOUND CYTOKINE STIMULATIONS

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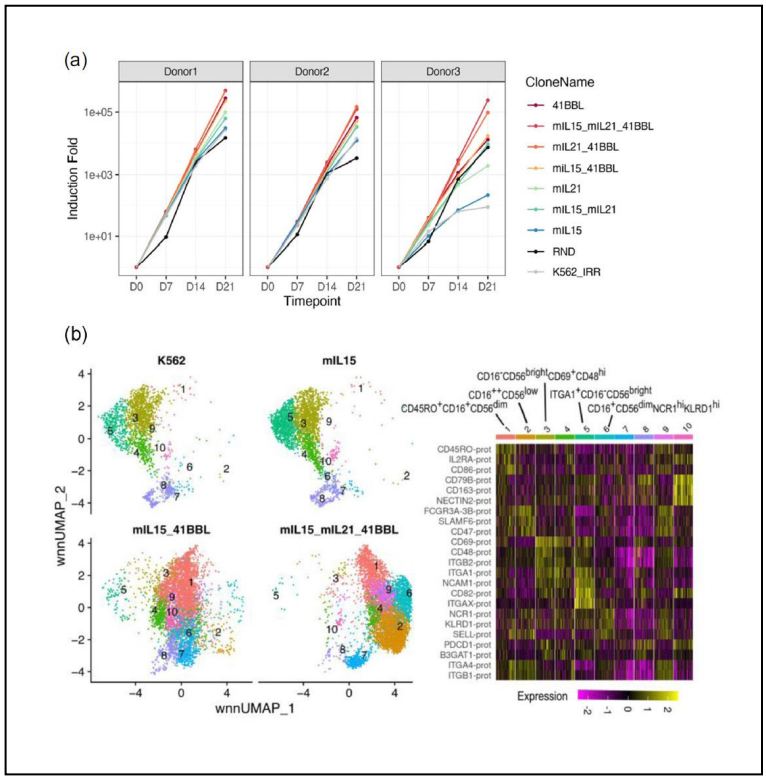
Robust manufacturing production of natural killer (NK) cells has been challenging in allogeneic NK cell-based therapy. Here, we compared the impact of cytokines on NK cell expansion by developing recombinant K562 feeder cell lines expressing membrane-bound cytokines, mIL15, mIL21, and 41BBL, individually or in combination. We found that 41BBL played a dominant role in promoting up to 500,000-fold of NK cell expansion after a 21-day culture process without inducing exhaustion. However, 41BBL stimulation reduced the overall cytotoxic activity of NK cells when combined with mIL15 and/or mIL21. Additionally, long-term stimulation with mIL15 and/or mIL21, but not 41BBL, increased CD56 expression and the CD56^{bright} population, which is unexpectedly correlated with NK cell cytotoxicity. By conducting single-cell sequencing, we identified distinct subpopulations of NK cells induced by different cytokines, including an adaptive-like CD56^{bright}CD16^{-}CD49a^{+} subset induced by mIL15. Through gene expression analysis, we found that different cytokines modulated signaling pathways and target genes involved in cell cycle, senescence, self-renewal, migration, and maturation in different ways. Together, our study demonstrates that cytokine signaling pathways play distinct roles in NK cell expansion and differentiation, which sheds light on NK cell process designs to improve productivity and product quality.



(a) NK cell expansion fold induction (y-axis in log10 scale) from three donors with indicated process conditions.

(b) Single cell analysis, TotalSeqC panel measured RNA expression and surface protein expression, reveals 10 NK cell clusters. Different process conditions drive NK cells to follow distinct differentiation trajectories, leading to divergence in cellular composition that affect the bioactivities of the cellular products.

Figure 1: Developing robust NK cell manufacturing processes with high yield and desired differentiation subsets