PD-L1 EXPRESSION IS INDUCED ON CYTOKINE-STIMULATED HUMAN NK CELLS AND CONTRIBUTES TO IFN-γ PRODUCTION

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Background: Natural Killer (NK) cells are critical effectors against tumor and virus-infected cells, and their activity is modulated by multiple inhibitory and activating receptors and cytokines. Tumor-experienced NK cells express high levels of PD-L1, and PD-L1$^+$ NK cells inhibit T cell priming. PD-L1 cell-intrinsic signaling pathways were described in tumor cells. The **objective** of this work was to study PD-L1 expression on cytokine-stimulated human NK cells and to evaluate PD-L1-mediated regulation of NK cell effector functions.
Methods: The expression of PD-L1 on human NK cells (CD56$^+$ CD3$^-$ cells) from peripheral blood of healthy donors (HD) was evaluated by flow cytometry (FC). Additionally, isolated NK cells were pre-stimulated for 18 hours with IL-12+IL-15+IL-18, or IL-15+IL-18, with or without an IFN-γ-neutralizing antibody (Ab), in some experiments K562 cells were added to the cultures for the last 4 hours, in the absence or in the presence of a PD-L1 blocking Ab. PD-L1 expression, intracytoplasmic IFN-γ and degranulation (CD107a expression) were evaluated on NK cells by FC.
Results: PD-L1 was expressed by a fraction of peripheral blood NK cells from all HD (24.10%± 9.55%; n=35), it was further up-regulated by IL-12+IL-15+IL-18 or IL-15+IL-18 ($p<0.01$, n=4), and was unaffected by IFN-γ neutralization. PD-L1 blockade partially inhibited IFN-γ production (but not degranulation) by NK cells. Accordingly, IFN-γ was preferentially produced by PD-L1$^+$ NK cells ($p<0.05$ vs PD-L1$^-$ NK cells, n=4).
Conclusions: Cytokine stimulation induces PD-L1 up-regulation on NK cells, PD-L1$^+$ NK cells are endowed with enhanced IFN-γ production, which is partially mediated through PD-L1 signaling.

Keywords: NK cells, IFN- γ, PD-L1

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