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Favorable *in vitro* effects of combined IL-12 and IL-18 treatment on NK cell cytotoxicity and CD25 receptor expression in metastatic melanoma patients

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Abstract

Background: As IL-12 and IL-18 have important immunostimulatory role the aim of this study was to investigate their *in vitro* effects on functional and receptor characteristics of NK cells and their subsets in healthy controls (HC) and metastatic melanoma patients (MM).

Methods: Peripheral blood mononuclear cells (PBMC) of HC and MM were stimulated with culture medium alone, medium supplemented with IL-12 (10 ng/ml), IL-18 (100 ng/ml) and their combination. NK cell activity was determined using radioactive cytotoxicity assay, while perforin, CD107a and pSTAT-4 expression, IFN-γ production and the expression of NKG2D, DNAM-1, CD161, CD158a/b, CD25, IL-12R beta 1/2 receptors on $CD3^{-}CD56^{+}$ NK cells and their $CD3^{-}CD56^{dim+}$ and $CD3^{-}CD56^{bright+}$ subsets were analyzed by flow cytometry. Cytokine induced level of DAP10 in PBMC was analyzed by reverse transcription polymerase chain reaction.

Results: IL-12 alone or in combination with IL-18 significantly induced NK cell activity and CD107a degranulation marker expression in MM and HC, while IL-18 alone did not have any effect in patients. The combination of IL-12 and IL-18 significantly increased mean fluorescence intensity (MFI) of IFN-γ in all NK cell subsets in HC and only in the bright subset in MM. MM that belong to M1c group with metastasis in liver and increased LDH serum values had significantly lower increase in NK cell cytotoxicity after combined IL-12 and IL-18 treatment compared to the patients in M1a and M1b categories. These results could be explained by decreased IL-12R expression and lower increase in pSTAT-4 and perforin expression in NK cells of M1c patients after IL-12 and combined IL-12 and IL-18 treatment. IL-18 alone significantly decreased NKG2D receptor expression and level of DAP10 signaling molecule in MM, while combined IL-12 and IL-18 increased the expression of CD25 on all NK cell subsets in HC and MM. Additionally, MM that belong to M1a + M1b group had significantly higher increase in CD25 receptor expression compared to the patients in M1c group.

Conclusions: The novel data obtained in this study support the use of IL-12 and IL-18 in combination for developing new therapeutic strategies for metastatic melanoma especially for patients with better survival rate and prognosis.

Keywords: IL-12/IL-18, Metastatic melanoma, NK cell cytotoxicity, NKG2D, CD25

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