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ORGINAL ARTICLE

Interleukin-4-induced natural killer cell antitumor activity in metastatic melanoma patients

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ABSTRACT. NK cells are important effectors of innate immunity that mount the first line of defense toward tumor growth. Interleukin-4 (IL-4) has recently been shown to regulate NK cell function, although its role in the regulation of NK cell function in cancer patients has not been clarified. The aim of this study was to investigate the effect of IL-4 on the function and the receptor characteristics of CD16-defined NK cells and their cytotoxic CD16bright and regulatory CD16dim subsets. Peripheral blood lymphocytes obtained from 36 metastatic melanoma (MM) patients treated for 18 h with 10 ng/mL IL-4 were evaluated for NK cell cytotoxicity using the radioactive 51chromium release assay. Expression of the activating receptors NKG2D and CD161, as well as the inhibitory receptors CD158a and CD158b, was analyzed on CD3-CD16+ NK cells and their subsets by flow cytometry. IL-4 induced significant in vitro enhancement of NK cell activity, as well as increased expression of the CD107a degranulation marker, by CD3-CD16dim NK cells. NKG2D expression was also increased on CD3-CD16+ cells by IL-4 with no alteration of the expression of CD161 and inhibitory KIR receptors. Although in vitro treatment with IL-4 increased both the expression of NKG2D and the cytotoxicity of NK cells, it had no detectable effect on the transcription of the TGF-β gene in NK cells of MM patients. The IL-4-induced NK cell cytotoxicity and increased activating NKG2D receptor expression may indicate an important antitumor effect of IL-4 with a potential application for immunotherapy of MM patients.

Key words: melanoma, IL-4, NK cells

INTRODUCTION

Melanoma is the most aggressive form of cutaneous neoplasm prone to formation of regional and distant metastasis [1]. However, due to the immunogenicity of this tumor, spontaneous remissions have been observed. In this sense, melanoma cells have been shown to be susceptible to NK cell lysis [2]. NK cells are important effectors of innate immunity and they shape the antitumor immune response by direct cytotoxicity as well as production of interferon gamma (IFN-γ) [3]. Tumor cell recognition by NK cells is regulated by the complex balance of signals derived from activating (NKG2D and CD161) and inhibitory (KIR) receptors [4]. NK cells are composed of two functionally distinctive subsets. $CD16^{bright}$ NK cell subset contains abundant perforin and granzyme granules and displays potent cytotoxic activity while $CD16^{dim}$ NK cell subset is characterized with pronounced ability to produce cytokines (IFN-γ, TNF, IL-10, IL-13, and GM-CSF) and has regulatory function [3, 4]. However, it has been shown that metastatic melanoma patients have multiple defects in immune response, including suppressed NK cell activity [5, 6]. In this sense, immunomodulating agents such as cytokines that enhance the cytotoxicity of NK cells have been included in the treatment of metastatic melanoma patients [7]. In particular, IFN-α and IL-2 have been administrated in the immunotherapy of melanoma for several decades. However, since the therapeutic effects of IFN-α and IL-2 are limited, many other cytokines have been investigated in this disease [8].

Interleukin 4 (IL-4), originally identified as a B cell activating factor, plays many roles in the regulation of adaptive and humoral immunity. IL-4 acts as a growth and survival factor for lymphocytes, stimulating the proliferation of activated B cells and T cells [9]. This cytokine is crucially involved in the balance between Th1 and Th2 immunological responses, promoting, after antigen challenge, the differentiation of naïve helper T cells into Th2 cells and consequently induces Th2 immunity [10]. It is well established that atopic diseases such as atopic dermatitis are associated with elevated level of IL-4. Interestingly, some studies have suggested a

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reduced risk of malignant melanoma among patients with atopic dermatitis [11]. Although, in general, the Th1 immune response is considered to be more effective than the Th2 response for antitumor immunity, some studies in mice show that IL-4 serves to prime Th1-associated, tumor-specific cytotoxic T lymphocytes (CTLs) [11]. Furthermore, it has been shown that NK cells also express IL-4Rα and that NK cell cytotoxic activity against tumors is, at least in part, regulated by IL-4 [12].

Since there are no or only scarce data regarding the effect of IL-4 on NK cell function in cancer patients, the aim of this study is to analyze NK cell cytotoxicity and receptor expression after *in vitro* administration of IL-4 in metastatic melanoma (MM) patients.

METHODS

Blood samples

Peripheral venous blood was obtained from 36 MM patients (stage IV according to the 8th modified AJCC/UICC staging system) at the time of diagnosis prior to chemotherapy [13]. The study was approved by the Ethical committee of Institute of Oncology and Radiology of Serbia and all patients have provided signed consent before inclusion in the study. The characteristics of MM patients enrolled in this study are listed in *table 1*.

Peripheral blood mononuclear cell (PBMC) isolation and in vitro *cultivation*

PBMCs were isolated from heparinized blood obtained from MM patients using Lymphoprep (Nypacon, Oslo, Norway) density gradient, centrifuged at 500 g for 40 min, and washed three times in RPMI 1640 culture medium (CM), (Sigma, St. Louis, MO) supplemented with 10% fetal calf serum (FCS) (Sigma). After washing, PBMCs were immediately used for functional, phenotypic, and molecular analysis.

PBMCs were further cultivated for 18 h in CM alone and in CM supplemented with IL-4 (10 ng/ml) (Becton Dickinson, San Jose, USA) in six-well plates at 37 °C and 5% CO_2 in a humid atmosphere.

Table 1
Patient characteristics.

		N (%)
Age		
	Range	28-69
	Median	51
Gender		
	Male	22 (61)
	Female	14 (39)
Primary tumor localization		
	Head and neck	5 (14|)
	Trunk	12 (33)
	Upper limb	3 (8)
	Lower limb	12 (33)
	Unknown	4 (11)
Metastases		
	Lung	12 (33)
	Liver	13 (36)
	Soft tissues	8 (22)
	Bones	6 (17)
	Suprarenal	2 (6)
	Spleen	2 (6)
	Retroperitoneum	2 (6)
	Lymph nodes	25 (69)

^{51}CHROMIUM RELEASE ASSAY FOR NK CELL ACTIVITY

NK cell activity of IL-4-treated PBMCs was determined using a standard cytotoxicity assay [14]. One hundred microliters of PBMCs, as effector cells, at a concentration of 4.0×10^6/ml of CM and two 1:1 dilutions were mixed with 100 µl of the erythromyeloid cell line K562, as target cells, at a concentration of 0.05×10^6/ml, prelabeled with radioactive ^{51}Chromium (Na_2CrO_4, As = 3.7 MBq, Amersham, UK), to form triplicates for 80:1, 40:1, 20:1, effector cell (E) to target cell (T) ratios (E:T). Cell supernatants were used for the determination of the amount of released ^{51}Chromium from the lysed K562 cells in a gamma counter (Berthold, FRG) and expressed in counts per minute (cpm). The mean percent cytotoxicity was calculated as described previously [5].

LDH release assay for NK cell activity

Evaluation of NK cell activity was also performed by modified lactate dehydrogenase (LDH) release colorimetric assay [15]. The assay was performed at the same E:T ratios of lymphocytes and tumor K562 cells as described previously. Briefly, 0.1 ml of PBMC as effectors cells were mixed with K562 cells as target cells. After a 2 h incubation and 5 min centrifugation at 200 g, 0.1 ml of supernatants were transferred into another flat-bottom 96-micro-well plate, and to each well 0.1 ml of the lactic acid dehydrogenase substrate (5.4×10^{-2}M L(+) Lactata, 6.6×10^{-4}M 2-p-iodophenyl-3p-nitrophenyl tetrazolium chloride, 2.8×10^{-4}M phenazine metrosulfate, and 1.3×10^{-3}M NAD in 0.2 M TRIS buffer, (pH 8.2) was added. Microplate absorbance spectrophotometer (Bio-Rad, USA) was used to read the absorbance at 490–630 nm. Percentage of cytotoxicity was calculated as previously described [15].

Flow cytometric analysis

Surface immuno-phenotype of IL-4-treated PBMC subsets were identified using the following combinations of directly labeled monoclonal antibodies (mAbs): CD3PerCP/CD16FITC, CD3PerCP/CD16 FITC/CD161PE, CD3PerCP/CD16FITC/CD158bPE, CD3PerCP/CD16PE/CD158aFITC (Becton Dickinson, San Jose, USA), and CD3PerCP/CD16FITC/NKG2DPE (R&D, Minneapolis, MN, USA). The samples were prepared as previously described [16]. Surface marker expression was quantified on FACS-Calibur flow cytometer (Becton Dickinson, USA). A total of 50 000 gated events, verified as peripheral blood lymphocytes according to their physical characteristics (FSC and SSC), were collected per sample and analyzed

using the CellQUEST software. Exclusion of nonspecific fluorescence was based on matched IgG1 isotype mAb combinations conjugated with FITC (clone X39), PE (clone X40), and PerCP (clone SK7), (Becton Dickinson, USA). NK cells were defined and gated within the lymphocyte gate according to their expression of CD3 and CD16 ($CD3^-CD16^+$). In order to define the two NK cell subsets of $CD16^{low}$, i.e., $CD3^-CD16^{dim}$ or $CD16^{high}$, i.e., $CD3^-CD16^{bright}$ subsets, $CD3^-CD16^+$ NK cells were divided based on the density of CD16 antigen defined by mean fluorescence intensity (MFI). NK cell receptors, NKG2D, CD161, CD158a, and CD158b were expressed as the percentage of gated $CD3^-CD16^+$ NK cells and their $CD3^-CD16^{dim}$ and $CD3^-CD16^{bright}$ NK cell subsets.

CD 107 degranulation assay

After 18 hour of *in vitro* cultivation 100 µl of PBMCs from MM patients (3×10^6cells/ml) were cocultured for 4 hours at 37 °C in a humidified atmosphere in a CO_2 incubator with 100 µl of suspension of K562 cell (2×10^6/ml) and with K562 cell (3×10^6/ml) suspension at 3:2 and 1:1, E:T ratios. Monensin (Sigma, USA) at a final concentration of 2 µl/ml was added after 1 hour incubation. Cells were afterward stained with CD16FITC, CD107aPE, and CD3PerCP mAbs (Becton Dickinson, USA). The percentage of CD107a was estimated by flow cytometry on gated $CD3^-CD16^+$ NK cells, as well as on $CD3^-CD16^{dim}$ and $CD3^-CD16^{bright}$ NK subsets. Spontaneous CD107a expression was determined in the absence of K562 cells.

Analysis of intracellular IFN- production

For measurement of intracellular IFN- production by IL-4-stimulated PBMCs (1×10^6/ml) from MM patients for the last three hours of *in vitro* cultivation brefeldin A was added at a concentration of 10 µg/ml. The cells were then stained for cell surface molecules CD3 and CD16, followed by fixing and permeabilizing with the Permeabilizing Solution 2 (Perm 2) (Becton Dickinson, USA) accords to the manufacturer's instructions. Then, the cells were stained with anti-IFN-γ FITC (Becton Dickinson). The expression of intracellular IFN-γ was analyzed in $CD3^-CD16^+$ NK cells and their $CD3^-CD16^{dim}$ and $CD3^-CD16^{bright}$ subsets.

NK cell isolation and culture

NK cells were purified from PBMCs by negative selection using the MojoSort Human NK cell Isolation Kit (Biolegend, USA), according to the manufacturer's protocols. The purity of NK cells was approximately 95% as measured by flow cytometry. Isolated NK cells were then cultured overnight in CM alone and CM supplemented with IL-4 (10 ng/ml) for gene expression analysis.

CFSE cell proliferation assay

Proliferation of isolated NK cells was monitored using dye dilution carboxyfluorescein diacetate succinimidylester (CFSE) assay. Briefly, cells were labeled independently with CFSE (Invitrogen, Life technologies Corporation, Eugene, Oregon) as follows: 2×10^6 isolated NK cells were incubated with 5 µM of CFSE diluted in DMSO for 20 min at 37 °C, washed extensively and were left overnight at 37 °C 5% CO_2 in CM alone and CM supplemented with IL-4 (10ng/ml). After cultivation CFSE labeled cells were collected in flow cytometry tubes and washed twice with 2 mL Phosphate Buffered Saline (PBS) (300 g for 5 min, at 4 °C) and resuspended prior to the analysis on FACSCalibur (BD, USA) flow cytometer.

qPCR amplification

For the purpose of quantitative PCR (qPCR), total RNA was extracted from IL-4- stimulated NK cells of MM patients using TRI Reagent® BD kit (Sigma) according to the manufacturer's recommendations. RNA bands were visualized on UV transilluminator and RNA concentration was determined spectrophotometrically (BioSpec Nano) (Shimadzu, Korneuburg, Austria). To prepare primary complementary DNAs (cDNA) with random primers by RT-PCR, 2 µg total RNA was used as template for MultiScribeTM Reverse Transcriptase in a High-Capacity cDNA Reverse Transcription kit (Thermo Fisher Scientific, Massachusetts, USA). The reverse transcription reaction was conducted according to the manufacturer's instructions.

All target transcripts were detected using quantitative Real time PCR (qPCR) and Taqman assays. TaqMan® Gene Expression Assay (*TGFB*-Hs00998133_m1) consists of 20X mix of unlabeled PCR primers and TaqMan® MGB probes (FAM^{TM} dye-labeled). Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) for which data was obtained using TaqMan control reagents (Hs02758991_g1) (Thermo Fisher Scientific) served as an endogenous control. PCR reactions were performed using an ABI Prism 7500 Sequence Detection System (Thermo Fisher Scientific). The PCR reaction conditions were described previously [17].

Statistical analysis

Significance of differences for obtained results was done by the nonparametric Wilcoxon signed rank test.

RESULTS

Standard chromium release assay performed using K562 tumor cells as targets shows that NK cell cytotoxic activity of investigated MM patients after 18 hour *in vitro* cultivation in culture medium decreases, although not statistically significant, compared to values obtained on samples of freshly isolated PBMCs. *In vitro* cultivation with 10 ng/ml of IL-4 (*figure 1A*) significantly ($p < 0.05$, Wilcoxon signed rank test) enhanced NK cell cytotoxicity compared to the control CM treatment (*figure 1A*). Moreover, the evaluation of NK cell cytotoxicity by the LDH release assay also showed significant ($p < 0.05$, Wilcoxon signed rank

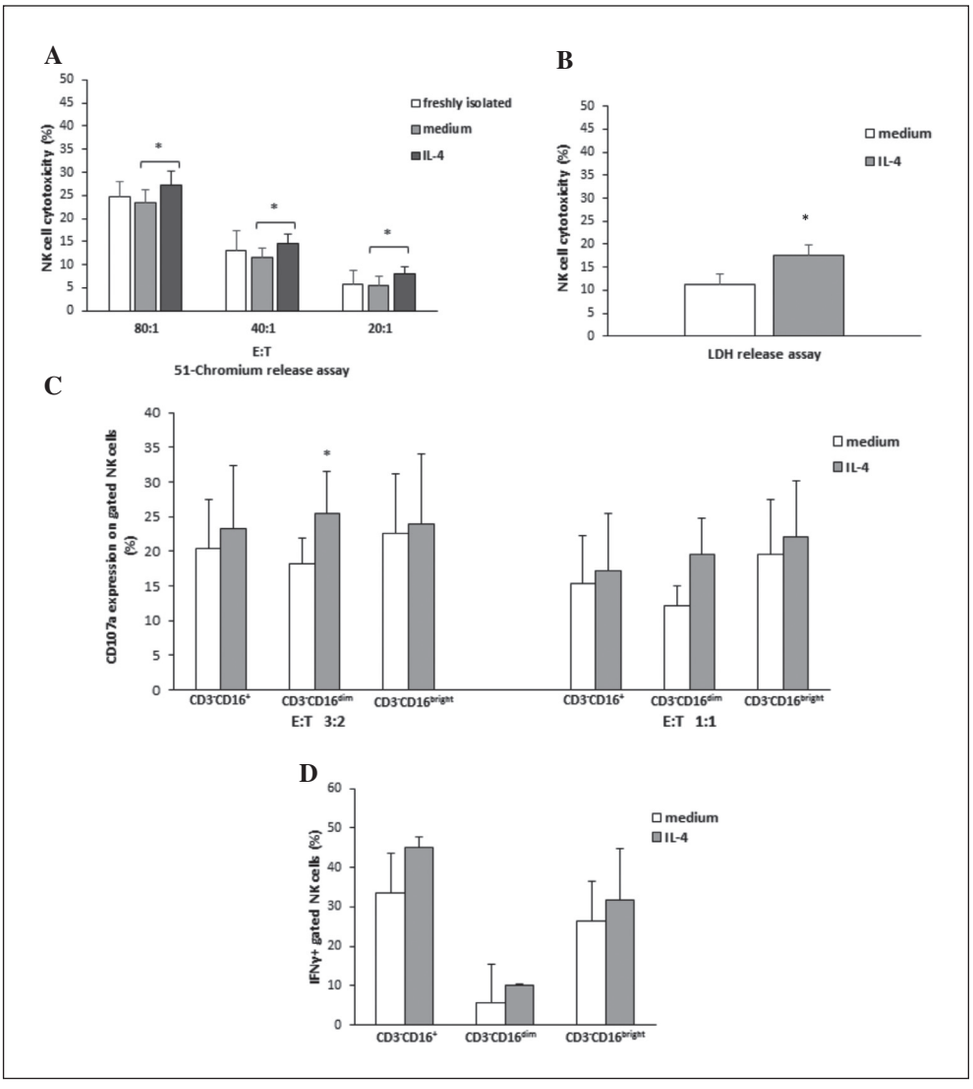

Figure 1

Natural killer (NK) cell function after treatment of peripheral blood mononuclear cells (PBMCs) of metastatic melanoma (MM) patients with 10 ng/ml of IL-4 for 18 hours *in vitro*. NK cell cytotoxicity against erythromyeloid K562 tumor target cell line: A) evaluated by ^{51}Chromium release assay at 80:1, 40:1, 20:1 effector to target cell (E:T) ratios show significant ($p \leq 0.05$, Wilcoxon signed rank test) increase after IL-4 compared to control cell culture medium treatment. Mean value for NK cell cytotoxicity for freshly isolated PBMCs is also shown; B) evaluated by LDH release assay at 50:1, E:T ratio shows significant ($p \leq 0.05$, Wilcoxon signed rank test) increase after IL-4 compared to control cell culture medium treatment; C) evaluated at 3:2 and 1:1, E:T ratios as the percentage of the CD107a degranulation marker on $CD3^-CD16^+$ NK cells and their dim and bright subsets show a significant ($p \leq 0.05$, Wilcoxon signed rank test) increase on the $CD3^-CD16^{dim}$ subset. D) Production of intracellular IFN-γ in NK cells and their dim and bright subsets in MM patients. The data are presented as mean ± standard error for 36 MM patients.

test) augmentation of NK cells activity after treatment with IL-4 (*figure 1B*).

Further analysis of NK cell function shows that IL-4 treatment also significantly ($p < 0.05$, Wilcoxon signed rank test) increases the percentage of the CD107a degranulation marker on the $CD3^-CD16^{dim}$ NK cell subset (*figure 1C*).

In contrast, the production of intracellular IFN-γ in NK cells and their dim and bright subsets does not change significantly ($p > 0.05$, Wilcoxon signed rank test) after *in vitro* treatment with IL-4 (*figure 1D*).

Treatment with IL-4 does not have any significant effect ($p > 0.05$, Wilcoxon signed rank test) on the percentage of NK cells and their dim and bright subsets in MM patients (*figure 2*).

The expression of NK cell receptors was analyzed on gated $CD3^-CD16^+$ NK cells and their $CD3^-CD16^{dim}$ and $CD3^-CD16^{bright}$ subsets after IL-4 treatment. The obtained results show a significant increase ($p < 0.05$, Wilcoxon signed rank test) of the expression of NKG2D activating receptor on NK cells (*figure 3A,B*), while for activating CD161 and inhibitory KIR receptors, CD158a and CD158b, there is no significant change ($p > 0.05$, Wilcoxon signed rank test) after cytokine treatment (*figure 3C*).

Flow cytometry data obtained on NK cells isolated from peripheral blood of MM patients and then labeled with CFSE, show that *in vitro* cultivation with IL-4 compared to the control culture medium only, did not induce proliferation of NK cells (*figure 4*).

The evaluation of the effect of IL-4 on *TGF-β1* gene expression in separated NK cells using Quantitative Real Time PCR (qPCR) shows that the expression of this gene does not change after 18 hours *in vitro* treatment with IL-4 (*figure 5*).

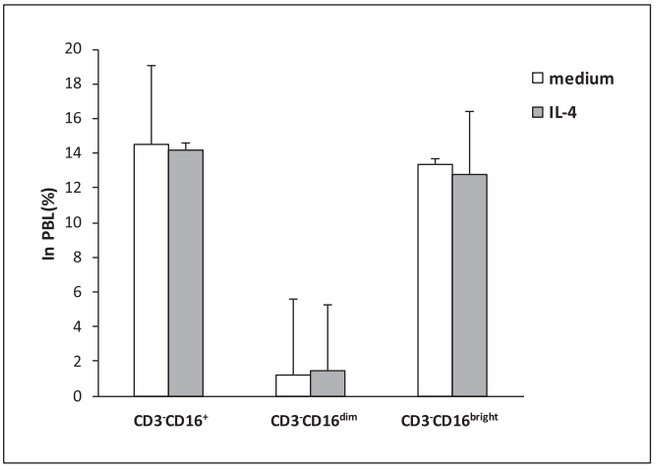

Figure 2
Effect of IL-4 treatment on the percentage of NK cells and their dim and bright subsets in cells from MM patients ($p > 0.05$, Wilcoxon signed rank test). The data are presented as mean ± standard error for 36 MM patients.

DISCUSSION

Aside from its well-known pro-Th2 effect, IL-4 is also able to regulate NK cell function. However, the effects of IL-4 on NK cells are not well established and the role of IL-4 in the regulation of NK cell function still needs to be elucidated.

It has been shown that NK cells in MM patients are functionally impaired with low antitumor cytotoxic activity and reduced ability to produce IFN-γ compared to healthy individuals [18]. We here demonstrate that in MM patients, by using the standard ^{51}Chromium release assay and the LDH release assay, the NK cell cytotoxicity is significantly augmented by *in vitro* treatment with IL-4. These data are also supported with the incresed expression of the CD107a degranulation marker on the $CD3^-CD16^{dim}$ NK cell subset induced with IL-4 treatment. The increasement of NK cell cytotoxic activity could be attributed to the effect of IL-4 on $CD3^-CD16^{dim}$ NK cell subsets. A similar effect of this cytokine on NK cell cytotoxicity was also obtained in helathy individuals (data not shown). It has previously been suggested that IL-4 directly or indirectly can affect NK cell function. Recent data obtained in experimental murine allergy models suggest that IL-4 mediates its tumor-suppressive effect via the activation of NK cell antitumor cytotoxicity [11]. These findings are supported by other *in vivo* studies where IL-4 produced by activated memory Th2 cells induced potent and long-lasting antitumor effects in mice through direct activation of NK cells [19]. Furthermore, *in vitro* stimulation of human PBMC with 1 ng/ml of IL-4 was shown to increase spontaneous cytotoxicity against K562 tumor cell line [20]. Indirect effect of IL-4 on NK cells cytotoxicity was also reported by Kiniwa *et al*. [12], showing that IL-4 overexpression induced proliferation of tissue-resident macrophages thereby contributing in NK cell activation via IL-15 production. Finally, it has been suggested that IL-4 activates NK cells by inducing the maturation of dendritic cells [21]. Contrary to the findings concerning the stimulatory effects of IL-4 on NK cell cytotoxicity, there are some reports indicating that IL-4 exposure may suppress the cytotoxic capacity of NK cells in both *in vitro* and *in vivo* models [22, 23].

In the present study, we show that the ability of NK cells to produce IFN-γ and exert an immunoregulatory function was not significantly upregulated after IL-4 stimulation, although there was a tendency of increased expression of IFN-γ in IL-4-treated NK cells of MM patients. This is in agreement with an in vivo study in mice that reported that there was no detectable IFN-γ production from murine NK cells after culture in the presence of IL-4 [24]. In contrast, Son *et al.* suggest that the anticancer activity of IL-4 toward melanoma cells is mainly mediated by upregulation of IFN-γ.

Furthermore, as we show in this study that IL-4 exposure *in vitro* had no detectable effects on the percentage of NK cells and their dim and bright subsets. These data may, together with data obtained by CFSE dye dilution assay, indicate that the enhancement of NK cell cytotoxicity observed with IL-4 treatment is not the consequence of NK cell proliferation (*figure 4*) but of increased cytotoxic capacity of NK cells treated with IL-4. In agreement, Nagler *et al* have reported that IL-4 inhibits the IL-2-induced proliferation of $CD16^+$ NK cells [25], the most abundant NK cell subtype, whereas a stimulatory effect of IL-4 on IL-2-induced proliferation was detected for the small population of $CD16^-$ NK cells [11].

NK cell cytotoxic function is regulated by the balance of signals derived from array of NK cell receptors and hence the impaired NK cell function in MM patients is often associated with reduced expression of activating NK cell receptors [5, 18]. The investigation of NK cell receptor expression shows that IL-4 significantly increases the expression of activating NKG2D receptor on NK cells in MM patients. Kiniwa *et al*. report that NK cells stimulated with IL-4 express activating NK cell receptors including NKG2D and NKp46 [12]. In apparent contradiction, it was reported that IL-4 treatment of mice can repress the expression of NKG2D receptor that, under resting conditions, is expressed on virtually all NK cells [23].

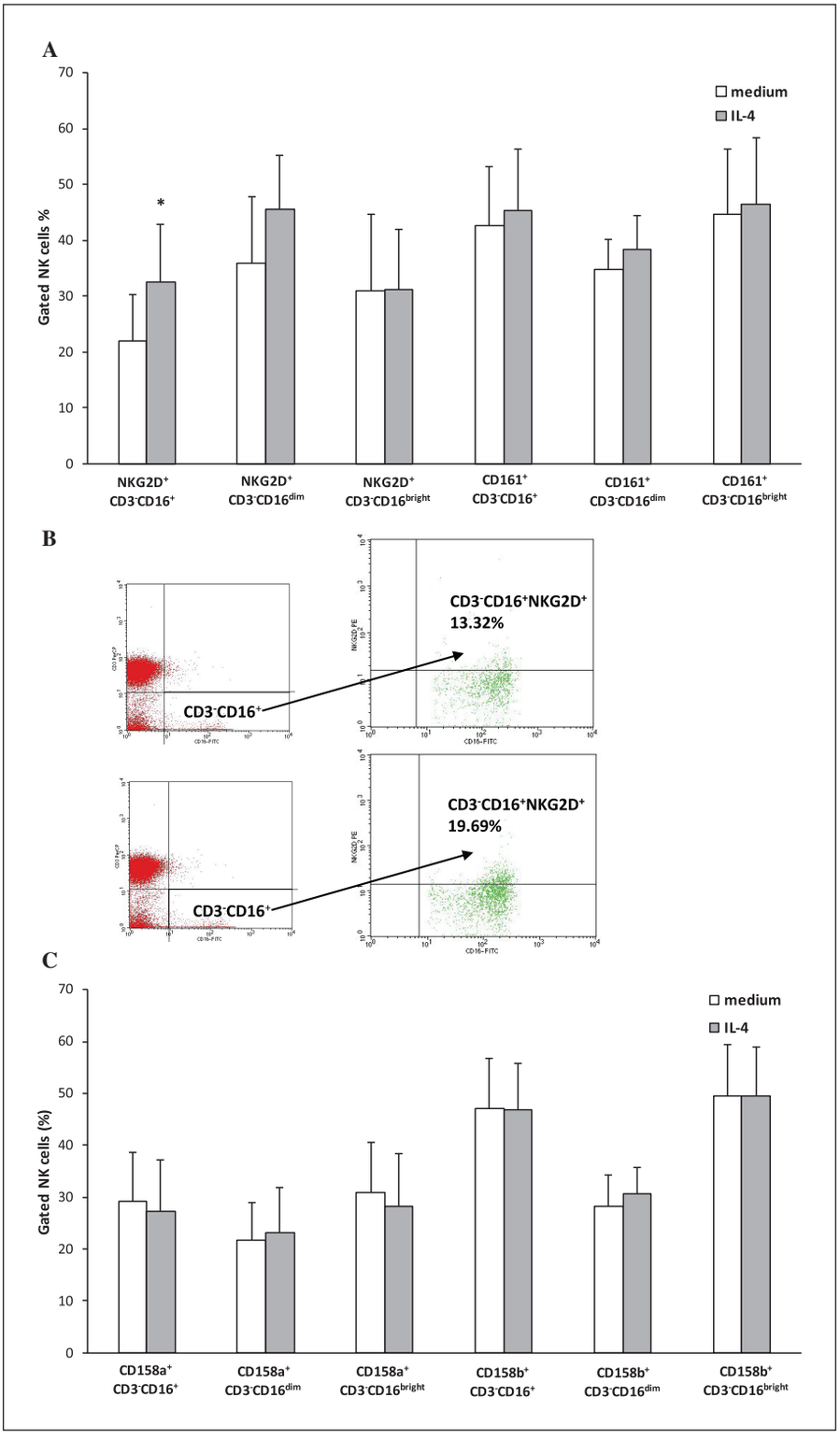

Figure 3

Expression of natural killer (NK) cell receptors on gated $CD3^-CD16^+$ NK cells and their $CD3^-CD16^{dim}$ and $CD3^-CD16^{bright}$ subsets after 18 hours treatment of peripheral blood mononuclear cells from metastatic melanoma (MM) patients with IL-4. A) Expression of activating (NKG2D and CD161) receptors shows significant ($p \leq 0.05$, Wilcoxon signed rank test) increase of NKG2D on NK cells; B) Representative flow cytometric dot plots showing IL-4-induced NKG2D receptor expression. C) Expression of inhibitory (CD158a and CD158b) receptors. The data are presented as mean ± standard error for 36 MM patients.

It has been established that within the tumor microenvironment, TGF-β produced by the tumor cells, as well as by various immunosuppressive cells including regulatory T cell (Treg), myeloid-derived suppressor cells (MDSC), and tumor-associated macrophages (TAM), decreases the expression of activating NKG2D receptor on NK cells. Furthermore, NK cells are also a major source of TGF-β which constitutively produced substantial amounts of TGF-β [26]. In this study, we find that IL-4 in *vitro* treatment did not affect TGF-β gene expression in NK cells isolated from MM patients. Therefore, although *in vitro* treatment with IL-4 increases the expression of activating NKG2D receptor and cytotoxicity of NK cells, it did not affect

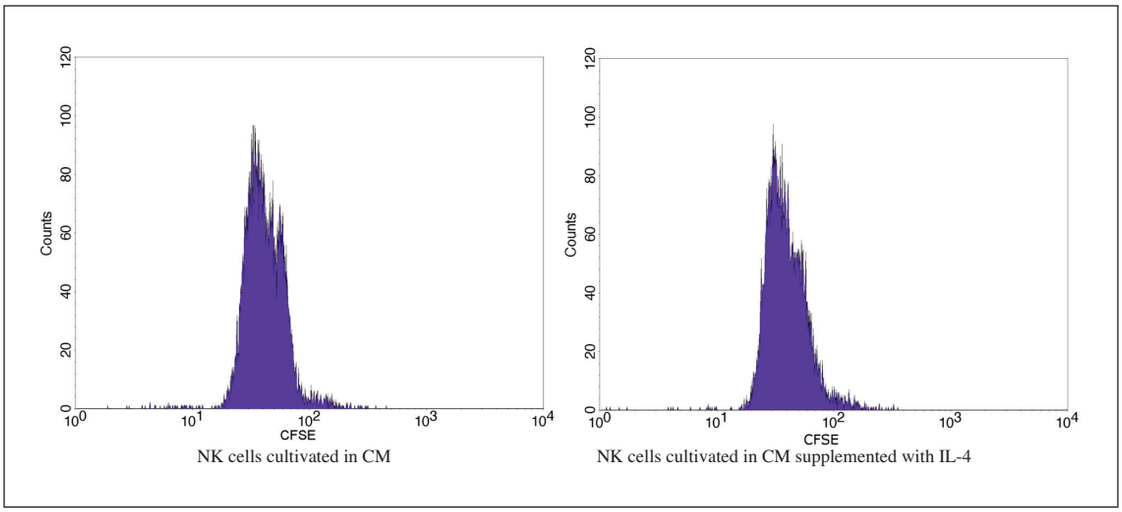

Figure 4
Representative flow cytometry histogram obtained of the carboxyfluorescein diacetate succinimidylester (CFSE) dye dilution assay showing no proliferation of NK cells after 18 hours of *in vitro* cultivation with cell culture medium (CM) only and CM supplemented with 10 ng/ml of IL-4.

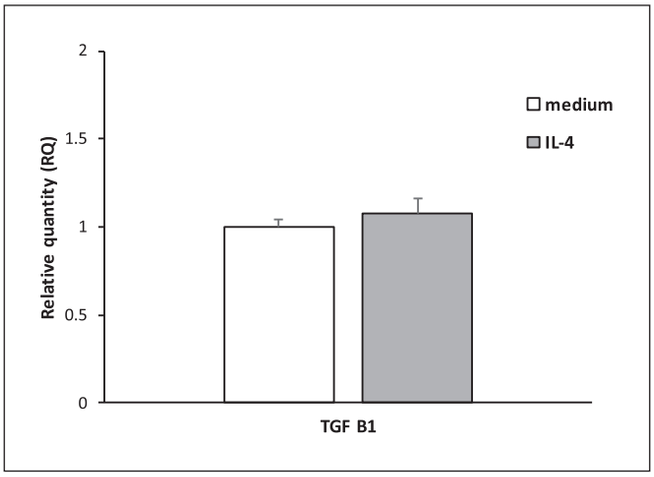

Figure 5
TGF-β1 gene expression in isolated NK cells after 18 hours treatment of IL-4 *in vitro* estimated by Quantitative Real Time PCR (qPCR) ($p > 0.05$, Wilcoxon signed rank test). The data are presented as mean ± standard error for 10 MM patients.

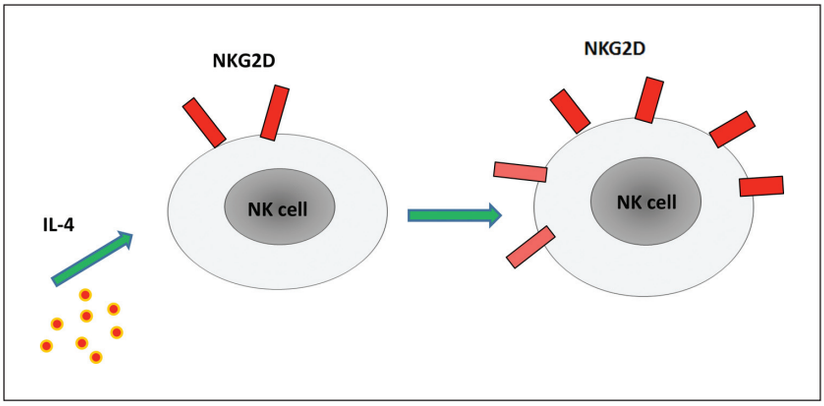

Figure 6
IL-4 increase expression of NKG2D receptor expression on NK cells in melanoma patients.

the transcription of the TGF-β gene in NK cells of investigated MM patients.

Contrary to the *in vitro* effect of IL-4 on the expression of NKG2D for activating CD161 and inhibitory KIR receptors, we show no change in their expression on NK cells. The lack of effect of IL-4 on the expression of CD158a/b is in agreement with previous reports [27].

It has been shown that in mice with IL-4-mediated experimental dermatitis, the proliferation of melanoma cells was inhibited through activation of $CD16^+$ NK cells that infiltrated the tumor tissue [11]. These data suggest that IL-4 produced in allergic diseases may induce an antitumor immune response by facilitating NK cell activity against tumor cells. Since CD16+ NK cells can be used for adoptive cell transfer immunotherapy, IL-4 that is produced by activated and memory Th2 cells [28] may also enhance NK cell cytotoxicity.

Although under physiological conditions, IL-4 contributes to the development of Th2-polarized immune responses [29]; this cytokine was shown to have antitumor effect in many tumors which were genetically modified to secrete IL-4. This may be due to the influence of IL-4 on the maturation of myeloid precursor cells that contributes to a more efficient activation of T and NK cells. Indeed, there are still many open questions regarding the roles of IL-4 in the antitumor immune regulation of different tumor types. In this study with MM patients we show a stimulatory effect of IL-4 on the antitumor function of NK cells. Therefore, similarly to many other cytokines, IL-4 may be considered a double-edged sword in antitumor immunity. Therapeutic modalities that utilize the beneficial and avoid the deleterious effect of IL-4 in cancer patients still remain to be clarified. However, IL-4 had shown encouraging antitumor activity in various experimental models in mice [30], which further stimulated clinical trials of this cytokine in cancer patients with different tumor types [31, 32].

In summary, we here show that IL-4 increases the effector function of NK cells including their cytotoxicity and the expression of activating NKG2D receptors in cells from MM patients (*figure 6*). We further suggest that IL-4-induced NK cells may have important antitumor effects that may be applicable for cellular therapy of metastatic melanoma patients.

CONFLICTS OF INTEREST

None declared.

Acknowledgments

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