In-vitro activation of natural killer cells from regional lymph nodes of melanoma patients with interleukin-2 and interleukin-15

Ana M. Vuletić[a], Irena P. Jovanić[a], Vladimir B. Jurišić[f], Zorka M. Milovanović[b], Srđan S. Nikolić[c], Nikola T. Tanić[d] and Gordana M. Konjević[a,e]

Regional lymph nodes (LNs) represent the first barrier in lymphogenic tumor dissemination in melanoma. Natural killer (NK) cells, the effector cell subpopulation of the innate immune system, are in the first line of antitumor immune defense. Therefore, the aim of this study was to investigate the effect of interleukin (IL)-2 and IL-15, two cytokines with similar immune-enhancing effects, on antitumor cytotoxic function and immunophenotype of NK cells from regional LNs of melanoma patients. Mononuclear cells purified from regional LNs of 50 melanoma patients in clinical stage II–IV were treated *in vitro* for 72 h and 7 days with 200 IU/ml rhIL-2 and 25 ng/ml IL-15 at 37°C in 5% CO_2. Both cytokines significantly augmented NK cell cytotoxic activity, transcription of the cytotoxic molecule perforin, and the level of functionally mature perforin in both nonmetastatic and metastatic regional LNs. IL-2 treatment increased the percentage of $CD3^-CD56^+$ NK cells by increasing the $CD56^{bright}$ NK cell subset in both nonmetastatic and metastatic LNs, whereas IL-15 treatment did not affect the percentage of NK cells and their subsets. Both cytokines increased on NK cells from nonmetastatic and metastatic LNs the expression of CD69 early activation antigen, the NKG2D activating receptor, as well as CD16 and inhibitory killer-cell immunoglobulin-like receptor CD158b, both inherent to the mature and the cytotoxic NK cell phenotype. In conclusion, our data may indicate the therapeutic potential of the NK cell population from regional LNs either as immunotherapeutic targets or as adoptively transferred after activation with IL-2 or IL-15. *Melanoma Res* 25:22–34

Melanoma Research 2015, **25**:22–34

Keywords: CD16, cytotoxicity, interleukin-15, interleukin-2, killer-cell immunoglobulin-like receptor, lymph nodes, melanoma, natural killer cells, NKG2D, perforin

Departments of [a]Experimental Oncology, [b]Pathology, [c]Surgical Oncology, Institute of Oncology and Radiology of Serbia, [d]Department of Neurobiology, Institute for Biological Research 'Siniša Stanković', [e]Department of Oncology, Faculty of Medicine, University of Belgrade, Belgrade and [f]Department of Pathophysiology, Faculty of Medicine, University of Kragujevac, Kragujevac, Serbia

Correspondence to Ana M. Vuletić, PhD, Department of Experimental Oncology, Institute of Oncology and Radiology of Serbia, 14 Pasterova, 11000 Belgrade, Serbia
Tel: +381 11 2067 289; fax: +381 11 2685 300;
e-mail: radovanovica@ncrc.ac.rs

Received 7 March 2014 **Accepted** 6 October 2014

Introduction

Cutaneous melanoma is a type of skin cancer with a very high mortality rate. As melanoma is an immunogenic tumor insensitive to irradiation and poorly sensitive to chemotherapeutic agents, immunomodulating agents have been introduced in melanoma therapy [1].

Natural killer (NK) cells, the effector cell population of the innate immune system, play an important role in antitumor immune response. NK cells can recognize stress-induced ligands on malignantly transformed cells without previous activation nor major histocompatibility complex (MHC) class I restriction and eliminate them by mechanisms of cytotoxicity. NK cells also produce abundant cytokines and chemokines and regulate adaptive and innate antitumor immune responses [2]. The cell surface phenotype that defines human NK cells within lymphocyte population is $CD3^-CD56^+$ [3]. Besides circulating in peripheral blood, NK cells are also present in secondary lymphoid organs, skin, liver, intestinal mucosa, lungs, uterus, etc. [4]. In melanoma patients, the NK cell immunophenotype and cytotoxic function have been evaluated in peripheral blood [5,6] and tumor tissue [7] and, only recently, in regional lymph nodes (LNs) [8].

NK cell cytotoxic activity against tumor cells can be modulated by various cytokines [9]. The important role of NK cell cytotoxic function in the activation and also in NK cell differentiation is mediated by the two γc-receptor family cytokines: interleukin (IL)-2 and IL-15. These two cytokines share the IL-2 receptor subunits β and γc, as well as the same signaling pathway, and hence have overlapping activities, but differ in their α subunit and therefore serve some self-specific functions. Both cytokines stimulate the growth, survival, and antitumor effector functions of NK and T cells [10]. On the basis of its biological activity, IL-2 has been used for more than two decades in the therapy of metastatic melanoma which, in some of treated patients, resulted in the induction of long-lasting reemission [1]. Therefore, IL-2 therapy represents the proof of the principle that activation of immune cells may result in tumor rejection and represents a milestone in cancer immunotherapy. However, this treatment has also shown adverse effects

0960-8931 DOI: 10.1097/CMR.0000000000000126