# DECREASED NK CELL CYTOTOXICITY AND INCREASED T REGULATORY CELLS FACILITATE PROGRESSION OF METASTATIC MURINE MELANOMA

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# SMANJENA CITOTOKSIČNOST NK ĆELIJA I POVEĆANJE REGULATORNIH T LIMFOCITA UBRZAVA METASTAZIRANJE MALIGNOG MELANOMA MIŠA

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Received / Primljen: 23.03.2012. Accepted / Prihvaćen: 08. 06. 2012.

## ABSTRACT

*Malignant melanoma is the most aggressive form of skin cancer. Metastatic dissemination in distant organs is one of the hallmarks of melanoma progression. Immunosuppression and tumour escape from immune surveillance are thought to be the major factors responsible for the establishment and progression of melanoma; however, the exact mechanisms leading to decreased anti-tumour immunity are not completely understood. We aimed to analyse the anti-tumour immune response during hematogenous metastasis using a B16-F1 metastatic melanoma model in C57BL/6 mice. At 21 days after tumour cell inoculation, rapid metastatic melanoma growth was observed, reflected through the increased incidence, number and size of metastatic colonies in the lungs (B16-F1). Phenotypic analyses of splenocytes revealed an increased percentage of $CD3^+$T cells, a markedly reduced percentage of $CD19^+$ B cells and an increased percentage and absolute number of $CD4^+Foxp3^+$T regulatory cells. The cytotoxic activities of total splenocytes and isolated NK cells were significantly decreased in tumour-bearing mice. Thus, the metastatic progression of melanoma in this model is associated with diminished NK cytotoxicity, which may be due to an increased expansion of suppressive $CD4^+Foxp3^+$ T regulatory cells in the spleen.*

**Keywords:** *B16-F1, malignant melanoma, metastasis, NK cells, T regulatory cells*

## SAŽETAK

*Maligni melanom je najagresivnija forma tumora kože. Diseminacija metastatskih ćelija u udaljene organe je glavna karakteristika progresije melanoma. Smatra se da su imunosupresija i izbegavanje imunskog nadzora glavni faktori odgovorni za uspostavljane metastaza, ali precizni mehanizmi odgovorni za oslabljen antitumorski imunski odgovor nisu u potpunosti razjašnjeni. U ovoj studiji, korišćenjem eksperimentalnog modela metastatskog melanoma (B16-F1) u C57BL/6 miševima analizirali smo antitumorski imunski odgovor u toku hematogenih metastatskih procesa. Dvadeset prvog dana nakon ubrizgavanja tumorskih ćelija detektovan je ubrzan rast metastaza malignog melanoma što se ogleda u povećanoj incidenci, broju i veličini metastatskih kolonija u plućima. Fenotipska analiza splenocita ukazuje na povećan procenat $CD3^+$T limfocita, značajno smanjene $CD19^+$ B limfocita i povećan procenat i apsolutan broj regulatornih $CD4^+Foxp3^+$T limfocita. Citotoksička aktivnost ukupnih splenocita i NK ćelija u slezini je statistički značajno smanjena u miševima kojima su ubrizgane ćelije malignog melanoma. Dobijeni rezultati u ovom eksperimentalnom modelu ukazuju da metastatskoj progresiji melanoma značajno doprinosi smanjena ubilačka sposobnost NK ćelija koja je najverovatnije posledica zabeležene ekspanzije imunosupresivnih regulatornih $CD4^+Foxp3^+$ T limfocita u slezini.*

**Ključne reči:** *B16-F1, maligni melanom, metastaze, NK ćelije, regulatorni T limfociti*

**UDK: 616-006.81-033.2 / Ser J Exp Clin Res 2012; 13 (3): 85-92**
**DOI: 10.5937/SJECR13-1726**

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