# Th-17 CELLS AS NOVEL PARTICIPANTS IN IMMUNITY TO BREAST CANCER

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# TH-17 LIMFOCITI, NOVI UČESNIK U IMUNSKOM ODGOVORU NA TUMOR DOJKE

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## ABSTRACT

*Breast cancer is a leading cause of cancer-related deaths among women worldwide. Tumour surveillance constitutes a process of recognising and modifying tumour development and involves both innate and adaptive immune systems. During the progression of malignancy, the immune response is dynamically changed. In our breast cancer model, we used 4T1 mouse mammary tumour cell lines with the capacity to metastasise efficiently to sites affected by human breast cancer. This model was used to evaluate anti-tumour immunity and tested **in vivo** whether tumour progression affected anti-tumour immunity. Female BALB/c mice were injected with 5 x $10^4$ 4T1 tumour cells into 4-th mammary fat-pad. Tumour size was evaluated daily and the number and size of tumour metastases was determined on day 36. Serum levels of pro-inflammatory cytokines, leukocyte cytotoxicity and cellular make up of the draining lymph nodes were tested in animals on day 13 after tumour inoculation. On day 36, metastases were found in the lungs and livers of the mice. IL-17 levels were higher in tumour bearing mice compared to healthy animals, while TNF-α serum levels showed no significant differences during tumour progression. Total cellularity of the draining lymph nodes was higher in tumour bearing mice. There were no differences in the total number of CD8+ and CD4+ cells; however, significant increases in CD19+ cells were found on the 13th day after tumour inoculation. Finally, MTT tests indicated higher cytotoxic activity levels in the draining lymph node cells of tumour bearing mice. We provide evidence suggesting that tumour induction may enhance immune responses most likely via the enhancement of Th-17 cells and the attenuation of CD4+Foxp3+ Treg cells.*

**Key words:** *mouse breast cancer, 4T1, metastasis, Th-17, Treg*

## SAŽETAK

*Tumor dojke je vodeći uzrok smrti kod žena širom sveta. Imunski nadzor predstavlja proces prepoznavanja i eliminacije malignih ćelija, koji uključuje i urođenu i stečenu imunost. Tokom progresije tumora, imunski sistem trpi dinamične promene. U ovom eksperimentu koristili smo 4T1 ćelijsku liniju mišjeg tumora dojke kao model tumora koji daje metastaze u organima zahvaćenim kod humanog karcinoma dojke. Cilj našeg istraživanja bio je ispitati efekte progresije tumora na anti-tumorsku imunost kod eksperimentalnog modela karcinoma dojke na BALB/C miševima. BALB/C miševima ženskog pola ubrizgano je 5 x $10^4$ 4T1 tumorskih ćelija direktno u masno jastuče mlečne žlezde broj 4. Veličina primarnog tumora merena je svakodnevno, a broj i veličina metastatskih kolonija 36-og dana erksperimenta. Trinaestog dana od ubrizgavanja tumorskih ćelija merili smo serumske nivoe pro-inflamatornih citokina, citotoksičnost leukocita i ćelijski sastav drenirajućih limfnih čvorova. Tridesetšestog dana od indukcije tumora, nadjene su metastaske kolonije na plućima i jetri. Izmeren je znatno viši serumski nivo IL-17 u miševima sa tumorima, a nije nadjena značajna promena u nivou TNF-α tokom progresije bolesti. Ukupna celularnost drenirajućih limfnih čvorova je povećana, 13-og dana nakon indukcije tumora. Nije pronadjena razlika u ukupnom broju CD8+ i CD4+ limfocita, ali je registrovano značajno povećanje ukupnog broja CD19+ limfocita. Ukupan broj CD4+Foxp3+ limfocita je značajno smanjen istog dana eksperimenta. Konačno, izmerena je veća citotoksična aktivnost tumor drenirajućih limfocita. Na osnovu navedenih rezultata, mi smatramo da indukcija tumora može da facilitira imunski odgovor, najverovatnije kroz aktivaciju Th-17 limfocita i smanjenje broja CD4+Foxp3+ T regulatornih limfocita (Treg).*

**Ključne reči:** *mišji tumor dojke, 4T1, metastaze, Th-17, Treg*

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