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THE CHARACTERISTICS OF CIRCULATING AND RESIDENT T-LYMPHOCYTES IN MICE WITH NEUROBLASTOMA

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The study presents the level of T-cell subsets in peripheral blood and secondary lymphoid organs in mice with neuroblastoma.

Keywords: T-lymphocytes, secondary lymphoid organs, flow cytometry, neuroblastoma.

The predominant role of T-lymphocytes in tumors is mediated by effector mechanisms, the production of cytokines and the regulation of other immune cells. Along with the main T-cells subsets ($CD3^+CD4^+$T-helpers and cytotoxic $CD3^+CD8^+$T-cells) double negative $CD3^+CD4^-CD8^-$T-cells constitute a rare population of peripheral T-cells and are of interest due to their poor investigated role in malignancies. DN T-cells possess both innate and adaptive immune functions differing from conventional $CD3^+CD4^+$ and $CD3^+CD8^+$T-cells, produce IFN-γ, TNFa, IL-4, perforin and granzyme B mediating the effect of destruction in malignant neoplasms [1].

The aim of the study was to characterize circulating and tissue resident T-lymphocytes in mice with neuroblastoma and control group.

The study materials were peripheral blood, spleen, lymph nodes and tumor tissue obtained from A/J mice with an experimental neuroblastoma model (group 1, n=5) and healthy mice (group 2, n=5). An experimental model of neuroblastoma was induced by subcutaneous injection of $1\text{-}2\times10^6$ cells of the NXS2 line and animals were euthanized on day 25-30 using a 2% solution of sodium thiopental. Immune cells were isolated from the spleen and lymph nodes by mechanical disaggregation followed by separation on a density gradient (ρ=1.087 g/cm^3, Carl Roth, Germany). Tumor-infiltrating lymphocytes (TIL) were obtained using automated mechanical dissociator for the preparation of cell suspensions (RWD, Russia) and TIL isolation kit (Miltenyi Biotec, Germany). The subpopulation composition of T-lymphocytes was evaluated by flow cytometry using mouse monoclonal antibodies CD3-FITC, CD45-PE, CD4-PC5, CD8-APC (Elabscience, China) and CytoFLEX S flow cytometer (Beckman Coulter, USA). Statistical data processing was carried out in STATISTICA 8.0 program.

Using flow cytometry analysis four subpopulations of circulating and tissue-resident T-lymphocytes were identified: $CD3^+CD4^+$T-helpers (Th), cytotoxic $CD3^+CD8^+$T-lymphocytes (CTL), double positive $CD4^+CD8^+$T-lymphocytes (DP) and double negative $CD4^-CD8^-$T-lymphocytes (DN). The number of T-cells subsets in investigated group is presented in table.

The number of T-lymphocytes in peripheral blood, spleen, lymph nodes and TIL in mice with neuroblastoma and control group, median (25÷75 percentiles)

Subsets	Groups	Peripheral blood	Spleen	Lymph node	TIL
$CD3^+CD4^+$Th, %	1	56.95 * (55.78÷6743)	60.91 * (59.94÷64.34)	53.20 (53.05÷55.63)	10.48 (4.70÷37.87)

	2	47.52 (33.90÷55.42)	46.00 (44.97÷57.17)	48.83 (40.43÷62.13)	
$CD3^+CD8^+$CTL, %	1	30.72 * (29.31÷41.20)	29.04 (20.33÷29.57)	28.57 (22.58÷42.90)	8.50 (7.05÷20.88)
	2	43.49 (33.57÷53.44)	27.49 (2382÷29,.20)	30.34 (14.98÷46.20)	
$CD4^+CD8^+$DP, %	1	0.24 (0.18÷0.99)	021 (0.16÷0.50)	0.76 (0.23÷0.97)	0.94 (0.06÷235)
	2	0.32 (0.15÷0.65)	0.46 (0.37÷0.78)	0.92 (0.69÷1.17)	
$CD4^-CD8^-$DN, %	1	2.83 * (2.61÷3.21)	10.34 * (7.88÷14.21)	11.62 * (3.72÷17.53)	81.00 (41.48÷85.63)
	2	10.69 (7.48÷15.39)	25.08 (17.67÷26.03)	6.44 (4.64÷29.78)	

Note: * - statistical significance of $p<0.05$ as compared with group 2.

The dominated subsets were $CD3^+CD4^+$Th in all investigated groups. The ratio of $CD3^+CD4^+$T-lymphocytes and $CD3^+CD8^+$T-lymphocytes (immunoregulatory index) was elevated in mice with neuroblastoma as compared with control group in the both peripheral blood (1.84 vs 1.09, $p<0.05$) and secondary organs: in the spleen (2.09 vs 1.67, $p<0.05$) and in lymph nodes (1.86 vs 1.61, $p<0.05$) that may indirectly be due to an increase in regulatory cells. While TIL composition in mice with neuroblastoma included only 10.48 (4.70÷37.87) % of $CD3^+CD4^+$Th and 8.50 (7.05÷20.88) % of $CD3^+CD8^+$CTL.

A statistically significant decrease of DN T-lymphocytes number in peripheral blood and spleen was found relative to the comparison group ($p<0.05$). At the same time, the analysis of lymph node cell suspension and TIL revealed an increase of DN T-lymphocytes relative to the control group ($p<0.05$). Herewith, the cellular composition of TIL in mice with neuroblastoma was consisted almost entirely of DN T-cells 81.00 (41.48÷85.63) % significantly differed from the level of DN T-cells in peripheral blood, spleen, lymph nodes ($p<0.05$). According to Zhiheng Wu et al., DN T-cells may secrete IL-10 with suppressive potential within mouse glioma and melanoma. The presence of DN TIL has also been demonstrated in lymph node metastases of melanoma patients, and a significant increase in DN TIL was found in the lymph nodes of melanoma patients who had disease progression compared to patients without it resulting in the hypothesis of DN TIL contribution to metastatic tumor progression [2].

Thus, the changes in circulating and tissue resident T-cells subsets composition in mice with neuroblastoma were revealed characterizing the predominance of DN T-cells among TIL and indicating their close relation to tumor formation and development.

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