INSTYTUT IMMUNOLOGII I TERAPII DOŚWIADCZALNEJ
im. L. HIRSZFELDA
POLSKA AKADEMIA NAUK

Bożena Ścirka

„Zróżnicowanie repertuaru receptorów antygenowych limfocytów T (TCR) w przebiegu eksperymentalnej immunoterapii mysiego czerniaka B16 przeciwciałem anty-GITR.”

„Diversity of the repertoire of T-cell antigen receptors (TCR) in the course of experimental immunotherapy of B16 melanoma with anti-GITR antibody."

Promotor:
dr hab. Arkadiusz Miążek

Wrocław 2019

SUMMARY

T-lymphocytes (T reg) are a subpopulation of T lymphocytes that suppress the immune response and induce tolerance to their own antigens. Inhibition of the immune response may also lead to a weakening of the anti-tumor response. A number of scientific studies confirm that the accumulation of these cells within the tumor promotes its growth. Therefore, an important goal of anti-cancer immunotherapy is selective inhibition of suppressor activity of T reg lymphocytes and at the same time promoting the activity of effector T cells, specific for tumor antigens. A promising strategy for immunotherapy tested on a mouse experimental model is stimulation of the GITR (glucocorticoid-induced TNFR-related protein - CD357) agonist monoclonal antibody DTA-1. The GITR molecule is a transmembrane type I protein with a length of 228 aa, belonging to the TNF family. It occurs in high density on the surface of T reg and activated T ef. *In vitro* and *in vivo* studies indicate that the simultaneous stimulation of GITR molecules and antigen receptors (TCR) suppresses the T suppressor function and affects T ef activation. The research undertaken in this study aims to identify how GITR binding by the DTA-1 antibody influences the clonal diversity and function of T cells specific for tumor antigens.

The research was based on the unique $TCR^{mini}Foxp3^{GFP}$ mouse model, in which the diversity of TCR receptors has been limited in order to be able to monitor changes in their repertoire at the level of individual, functionally separate clones, specific for tumor antigens. Thanks to this, it is possible to fully characterize TCR receptor repertoires on various lymphocyte subpopulations involved in the antitumor response. In TCR^{mini} mice, all of which have the same transgenic TCRβ chain (Vβ14Dβ2Jβ2.6), the diversity of TCRα chains limits the rearranging mini locus comprised of a single Vα2.9 segment and two Jα segments (Jα26 and Jα2). These mice develop normally functioning T-cell subpopulations with a reduced, polyclonal TCRαβ receptor repertoire. In addition, $TCR^{mini}Foxp3^{GFP}$ mice possess a genetic T-cell marker reg ($FoxP3^{+}$) in the expression of green fluorescent protein (GFP^{+}). Some of the preliminary experiments were also performed on $FoxP3^{GFP}$ transgenic mice. They possess the above-described label of T regulatory cells in the context of a non-concentrated TCR repertoire of mice, strain C57Bl / 6 (B6).

As a model tumor, a murine B16 melanoma line, genetically modified for the expression of neo-antigen (Ep63K peptide), was used. This neo-antigen is presented on the surface of B16 melanoma cells in the context of MHC class II-Ab, (II-AbEp63K). Previous work has demonstrated that the B16 melanoma line modified this way after subcutaneous implantation into $TCR^{mini}Foxp3^{GFP}$ transgenic mice activates at least 20 different TCR receptor T effector $CD4^+$ cells. The frequency of occurrence and presence among various T-cell populations of the mentioned receptors gives the possibility of insight into the course of the anti-tumor response and its modulation due to the administration of the DTA-1 antibody. During the project implementation an assessment was made:

- influence of anti-GITR immunotherapy on suppression of tumor growth kinetics,
- influence of anti-GITR immunotherapy on changes in the T reg and T ef cell populations during tumor development,
- analysis of sensitivity of T ef cells to the suppressive action of T reg isolated from tumors and lymph nodes draining during disease development and therapy with DTA-1 antibody,
- the impact of anti-GITR immunotherapy on the TCR receptor repertoire T reg and T ef cells in response to cancer.