ImmunoTools *special* Award 2021

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Blockade of the CD73 immune checkpoint on tumor-derived exosomes

Exosomes are endosome-derived nanoscale membrane vesicles that are actively secreted by numerous cell types, including malignant tumor cells. Tumor cell-derived exosomes (TEX) are known to carry a unique repertoire of cancer-associated (cell surface) proteins and mediators, which can promote oncogenic effects, including enhanced angiogenesis, proliferation, and metastatic spread. Additionally, emerging evidence indicates that TEX expose various immunoinhibitory molecules that allow cancer cells to escape from elimination by immune surveillance. Recently, it was shown that a significant part of the immunoinhibitory activity of TEX is attributable to ectonucleotidase CD73. CD73 is involved in down-regulation of pro-inflammatory immune responses by sequentially dephosphorylating extracellular ATP into adenosine (ADO), which is one of the most powerful immunosuppressive molecules in the human body. In this process, CD73 is rate-limiting in the conversion of adenosine monophosphate (AMP) to ADO. This catalysis step results in a rapid local increase of ADO, which subsequently engages the immunosuppressive actions of adenosine A2A and A2B receptors on locally present immune cells. Normally, this process provides a self-limiting and counterbalancing mechanism to timely and locally resolve the immune response.

Nanoscale vesicles like TEX can easily propagate to tumor-draining lymph nodes and reach the systemic circulation. Therefore, TEX-exposed CD73 may be involved in both intra- and extratumoral down-modulation of anticancer immune responses. In this respect, selective blockade of the CD73-immunecheckpoint on TEX may be useful to effectively overcome ADO-mediated immunosuppression in cancer.

It has been shown that blockade of immune checkpoints on cancer cells is a promising approach to overcome immunosuppression. In this respect, CD73-blocking antagonistic antibodies or small molecule inhibitors have been developed to selectively block the enzyme capacity of CD73 to convert AMP in to ADO. Consequently, blockade of CD73 enzyme activity may restore the anticancer activities of various immune effector cell types. However, the capacity antagonistic antibodies or small

molecule inhibitors to inhibit CD73 present on TEX has not been evaluated and is therefore topic of the proposed study.

A detailed evaluation of the CD73-adenosine immune checkpoint on TEX may be provide novel data that can be useful for the development of an alternate or complementary approach for current forms of cancer immunotherapy.

Hypothesis – We hypothesize that enhanced blockade of TEX-exposed CD73 by rationally designed antagonistic antibodies or small molecule inhibitors can be of therapeutic value.

Aim of project – We aim to evaluate the enzyme activity of CD73 on TEX and preclinical evaluate whether blockade of CD73 on TEX by antagonistic CD73-targeting antibodies or small molecule inhibitors can be of additional therapeutic potential.

The ImmunoTools antibodies will be a very useful tool to evaluate the possible modulatory effects of TEX-exposing CD73 on cancer- and immune- cell function and phenotype by flow cytometry. In particular, ImmunoTools has many antibodies that can be used for the identification of TEX (**CD9-FITC, CD63-APC**), different T-cells populations (**CD3-FITC, CD4-FITC, CD8-APC**), B-cells (**CD19-FITC, CD20-APC**), but also other immune cells like macrophages (**CD14-FITC, CD40-APC**). Furthermore, CD73-mediated suppression of the cancer cell killing capacity of immune cells will be monitored by flow cytometric staining for **Annexin-V-FITC**.

In conclusion, the ImmunoTools award would greatly contribute to my research by gaining insight into cancer cells and TEX-mediated immune suppression and evaluating the therapeutic potential of CD73-blocking antibodies.

ImmunoTools *special* AWARD for **Emily Ploeg** includes 10 reagents

FITC - conjugated anti-human CD3, CD4, CD9, CD14, CD19, Annexin-V

APC – conjugated anti-human CD8, CD20, CD40, CD63

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