ImmunoTools *special* Award 2024

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Determination of protein glycosylation profile of immune cells and influence of protein glycosylation on the functioning of immune cells

A vast majority of proteins synthesised by cells are subject to post-translational modifications, among which glycosylation is the most common. It is a process of attaching carbohydrates either to amino or hydroxyl groups of certain amino acids in protein, which takes place in endoplasmic reticulum and Golgi apparatus. Glycosylation influences the conformation of the proteins, their expression (both on the cell membrane and in the secreted form), stability, half-life, activity and antigenicity. Removing of carbohydrate groups from proteins may lead to their internalisation, degradation and, finally, reduced expression on the surface of the cell, which makes it a crucial modification required for the proper functioning of the proteins, cells and organisms.

Protein glycosylation patterns may vary between tissues and are highly influenced by various conditions, including cells' age, maturity, activation, metabolite availability, inflammatory mediators, miscellaneous diseases (e.g. infectious and autoimmune diseases as well as cancer), sex and even pregnancy and vaccinations.

Protein glycosylation influences immune system functioning by modifying protein conformation and also affects peptide loading on MHC (which regulates antigen presentation), clustering of the receptors on cell membrane (including TCR clustering, which is crucial for TCR signaling) and cell migration. Glycosylation regulates binding of the receptors and ligands, which is crucial for cell signaling and regulating cell functions. Glycosylation strongly impacts antibody functions, as changes in antibodies' glycosylation patterns may alter the nature of the reaction triggered in the responding cells.

The research on protein glycosylation profile of specific immune cell populations in vivo and in vitro is still rather insufficient. We also lack information about changes of

this glycosylation profile on immune cells associated with their functions in the immune system.

In my current work, I have determined protein glycosylation profile on the most important cell populations of adaptive immunity. I was able to show changes in glycosylation profile associated with the functioning of these cells. These findings support the importance of protein glycosylation in the functioning of the immune system and its regulation.

Despite these insightful results, there are still a few areas I would like to examine, in which ImmunoTools Special Award will be of great help. I would like to expand my research and find out more about protein glycosylation profile on the cells of innate immunity – monocytes and macrophages, including macrophages M1 and M2. Knowing the importance of the cells of innate immunity as the first line of defence, it is crucial to determine their protein glycosylation profile and its changes associated with the functioning. My preliminary results indicate that the cells of innate immunity might differ from the cells of adaptive immunity, when it comes to protein glycosylation. Moreover, M1 and M2 macrophages have different functions and participate in different processes in immune system. I want to examine their protein glycosylation profile and find out if there are any differences between these populations, which may relate to their functions.

In order to reach these objectives, I plan to identify different subpopulations of monocytes: classical, non-classical and intermediate by their surface markers (CD14, CD16, CD40, CD192 and HLA-DR) and determine their glycosylation profile. Then, I would like to differentiate monocytes into M1 and M2 macrophages using recombinant GM-CSF and IFN-γ (for M1) and IL-10 (for M2). I will identify these subpopulations by their surface markers: CD68, CD80 and CD163 and examine their glycosylation profile. To examine the interplay between functioning of the cells and protein glycosylation, I would like to measure the secretion of IL-6 and IL-12 in cell cultures.

ImmunoTools *special* AWARD for **Maria Piórkowska-Rokita**

includes 10 reagents

FITC - conjugated anti-human CD16

PE - conjugated anti-human CD68, HLA-DR, IL-6

PerCP - conjugated anti-human CD14

APC - conjugated anti-human CD40, CD80

anti-IL-12p40 purified

recombinant human cytokines: rh IL-10, rh IFN-γ

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