ImmunoTools *special* Award 2021

Balázs Gyebrovszki, PhD-student

Supervisor: Prof. Gabriella Sármay

Department of Immunology,
Eötvös Loránd University, Budapest, Hungary

The role of n-glycosylation of IgG Fc region in the pathomechanism of rheumatoid arthritis.

The site-specific n-glycosylation of IgG is a post-translational modification of the protein, which is responsible for the modulation of its effector function. It was observed that the hyposyalisation of the glycan in the Fc region enhances the inflammatory response.[1] However, the mechanism behind this is not clarified yet. Individuals suffering from rheumatoid arthritis (RA), an inflammatory autoimmune disease, produce several autoantibodies, which target proteins of the synovium and cartilage, terminally leading to severe bone erosion via osteoclast activation. More than 70 % of RA patients produce anti-citrullinated protein/peptide antibodies (ACPA) that have diagnostical and pathological significance.[2,3] Approximately 1% of the world's adult population suffers from this disease, with a three-fold higher likelihood of occurrence among women. The treatment of such patients mainly consists of administering disease modifying antirheumatic drugs (DMARDs) and biological therapy such as TNFα inhibitors like infliximab.

In our investigations we purified IgG from serum samples of healthy donors and RA patients. From the RA IgG samples we affinity purified ACPA using immobilized citrullinated multiepitope peptide. Then the IgG from both groups was dimerized with a $F(ab')_2$ crosslinking two IgG molecules by anti-human $F(ab')_2$ to form an artificial immune complex, which were then added to previously PMA-activated U937 cells.

This cell line was used as a model of monocytes/macrophages, a major source of inflammatory cytokines. It was observed that the dimers of both ACPA and control IgG bound to the Fcɣ receptors type I (FcɣRI) on the cell surface, but there was a significant difference in the FcγRI induced inflammatory cytokine release between the two groups. Namely, the addition of ACPA dimers triggered a significantly higher TNFα production as determined from the supernatants using a sandwich ELISA system. The MS-spectrometry analysis of the trypsin-digested glycopeptides from the samples determined the most abundant glycoforms from each group. It was revealed that there is a difference in the most abundant glycoforms from each group, in the healthy group the glycan bearing one galactose (which harbours the terminal sialic acid) was the most abundant, while in the RA samples the glycoform lacking galactose appeared to be of highest frequency. These results are consistent with the literature in this field.

In our next experiments we are planning to repeat the functional assays (cytokine release tests) with a Siglec-9 (sialic acid binding lectin 9) knockout variant of the cell line used, to determine said receptor's role in the inflammatory process. We also plan to survey primary monocytes/macrophages isolated from peripheral blood of healthy blood donors as well, for this endeavour we plan to utilize a stimulation with GM-CSF and an intracellular staining of TNFα, IL-6, IL-12, and IL-23 while staining the CD14 monocyte marker on the cell surface. IL-12 and IL-23 have a role in altering glycoslyation of IgG [1], if the binding of ACPA has an enhancing effect on the said cytokine's production, it may lead to the downregulation of the syalyl-transferase enzyme responsible for attaching terminal syalic acid to the glycan, leading to less syalylated IgG, increasing its pathogenicity.[4]

References:

1. *Coutant F, Miossec P. Evolving concepts of the pathogenesis of rheumatoid arthritis with focus on the early and late stages. Curr Opin Rheumatol. 2020 Jan;32(1):57-63. doi: 10.1097/BOR.0000000000000664. PMID: 31644463.*
2. *Hans Ulrich Scherer, Thomas Häupl, Gerd R. Burmester,The etiology of rheumatoid arthritis,https://doi.org/10.1016/j.jaut.2019.102400.*

3. *Toes R, Pisetsky DS. Pathogenic effector functions of ACPA: Where do we stand? Ann Rheum Dis. 2019 Jun;78(6):716-721. doi: 10.1136/annrheumdis-2019-215337. Epub 2019 Apr 20. PMID: 31005898.*
4. *Bordron A, Morel M, Bagacean C, Dueymes M, Pochard P, Harduin-Lepers A, Jamin C, Pers JO. Hyposialylation Must Be Considered to Develop Future Therapies in Autoimmune Diseases. Int J Mol Sci. 2021 Mar 26;22(7):3402. doi: 10.3390/ijms22073402. PMID: 33810246; PMCID: PMC8036829.*