BIO_05 - CLEC5A expression can be triggered by spike glycoprotein and may be a potential target for COVID-19 therapy

Thiago Lazari Machado[1]; Alanna Calheiros Santos[2]; Tamiris Azamor[1]; Andrea Marques Vieira da Silva[1]; Luciana Neves Tubarão[1]; Rodrigo Muller[1]; Marcelo Alves Pinto[1]; Livia Melo Villar[2]; Ana Paula Dinis Ano Bom[1]; Juliana Gil Melgaço[1].
[1]Fiocruz/Bio-Manguinhos
[2]Fiocruz/Instituto Oswaldo Cruz

Introduction: The immune response is crucial for coronavirus disease 19 (COVID-19) progression, with the participation of proinflammatory cells and cytokines, inducing lung injury and loss of respiratory function. CLEC5A expression on monocytes can be triggered by viral and bacterial infections, leading to poor outcomes. SARS-CoV-2 is able to induce neutrophil activation by CLEC5A and Toll-like receptor 2, leading to an aggressive inflammatory cascade, but little information is known about the molecular interactions between CLEC5A and SARS-CoV-2 proteins.

Objectives: Here, we aimed to explore how CLEC5A expression could be affected by SARS-CoV-2 infection using immunological tools with *in vitro*, *in vivo* and *in silico* assays.

Methodology: Molecular docking modeling was performed through the ClusPro 2.0 and Pymol 2.5 software. PBMCs were subjected to assays ex vivo immunophenotyping with commercial antibodies to characterize the monocyte subpopulations and Clec5a expression. The PBMC were isolated by Ficoll-Paque® and analyzed by flow cytometry. The samples were divided into three groups: unexposed (n=18), mild COVID- 19 (n=17) and severe COVID-19 (n=10). Quantification of the cytokines IL-2, IFN-γ, IL-6, and IL-1β was performed using an in-house multiplex liquid microarray test. Detection of CLEC5A gene expressed in blood from hamsters was performed by RT-qPCR. Blood samples were obtained at days 3, 5, 10, and 15 through exsanguination by cardiac puncture from a 36 Syrian golden hamster (Mesocricetus auratus) at 1 year of age and 150 ± 1.4 g infected intranasally with SARS-CoV-2 strains Delta (1.0 × 106 PFU/ml) and Omicron (1.0 × 106 PFU/ml).

Results: The findings revealed that high levels of CLEC5A expression were found in monocytes from severe COVID-19 patients in comparison with mild COVID-19 and unexposed subjects, but not in vaccinated subjects who developed mild COVID-19. In hamsters, we detected CLEC5A gene expression during 3-15 days of Omicron strain viral challenge. Our results also showed that CLEC5A can interact with SARS-CoV-2, promoting inflammatory cytokine production, probably through an interaction with the receptor binding domain in the N-acetylglucosamine binding site (NAG-601). The high expression of CLEC5A and high levels of proinflammatory cytokine production were reduced in vitro by a human CLEC5A monoclonal antibody.

Conclusion: CLEC5A was triggered by spike glycoprotein, suggesting its involvement in COVID-19 progression; therapy with a monoclonal antibody could be a good strategy for COVID-19 treatment, but vaccines are still the best option to avoid hospitalization/deaths.

Keywords: Clec5a, COVID-19, monocytes