ORT_03 - Effects of re-exposure to SARS-CoV-2 on cellular immune response and pulmonary cell lines: the perspective of an *in vitro* model

Juliana Gil Melgaço[1]*; Tamiris Azamor da Costa Barros[1]; Tiago Pereira dos Santos[1]; Thyago Leal Calvo[2]; Andréa Marques Vieira da Silva[1]; Natalia Fintelman-Rodrigues[3]; Aline Matos[2]; Marilda M. Siqueira[2]; Milton Ozório Moraes[2]; Ana Paula Dinis Ano Bom[1].
[1]Fiocruz/Bio-Manguinhos;
[2]Fiocruz/IOC;
[3]Fiocruz/CDTS.

Introduction: The new coronavirus (SARS-CoV-2) is causing the COVID-19 pandemic, widespread worldwide and without effective treatment. Vaccination has been started, but the total protection is under investigation. Viral reactivation and reinfection have been described, but data about the cellular immune response in a second exposure still unclear.

Objective: Here, we were aimed to assess the cellular immune response of the SARS-CoV-2 infection-induced or spike protein-stimulation in peripheral blood mononuclear cells (PBMC) from human unexposed and individuals with history of COVID-19 (primo-infected). Moreover, the supernatants of these cultures were used to evaluate the *in vitro* effects in pulmonary alveolar epithelial cell line (A549).

Methodology: PBMC were cultivated in 24 and 48 hours with viral antigens. The pool of the supernatants from PBMC infected with virus was cultivated with A549 cells during 48 hours. We used the current immunological methods, as flow cytometry and gene expression, to evaluate the SARS-CoV-2 cellular immune response and their effects in a pulmonary alveolar epithelial cell line (A549).

Results: Results showed that lymphocytes B and T, monocytes and natural killer cells were affected by viral replication (expressing double-strand RNA), however, natural killer cells presented a slightly reduction of viral replication after 48 hours in comparison with 24 hours of SARS-CoV-2 infection. Also, our data demonstrated that SARS-CoV-2 infection induced a strong antigen-specific immune response in COVID-19 individuals, mainly by CD4 memory T cells, with high expression of *IFNG*, and other genes related to inflammation and antiviral response. Among unexposed individuals, the innate cells (natural killer and monocytes) were induced predominantly after virus infection. The products in the supernatant of PBMC from unexposed subjects infected by SARS-CoV-2 were able to elevate apoptosis of alveolar epithelial cell lines (A549). Meanwhile, the supernatant's products of primo-infected PBMC after a re-exposure to SARS-CoV-2 contributed to reduce apoptosis and to elevate the antiviral activity (iNOS) of A549 cells.

Conclusion: Our findings suggest that second exposure to virus may be controlled by antiviral responses produced by immune system cells. Findings using an *in vitro* model also reinforce that cellular immune response is the key to a better understanding of the virus-host interactions during COVID-19, helping further studies focused on immunotherapies and vaccine development.

Keywords: immune response; SARS-CoV-2; cell cultivation