SARS-COV-2 CORONAVIRUS ANTIGENS MODULATE THE ACTIVITY OF HUMAN T-REGULATORY CELLS

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Regulatory T cells (Treg cells) play a crucial role in regulation of immune response. It is believed that excessive activity of Treg cells in the early stages of infectious diseases reduces the pro-inflammatory activity of cells in the innate and adaptive immune response, thereby contributing to the development of chronic inflammation or excessive inflammation. Additionally, it is known that aging leads to changes in the quantity of Treg cells and disrupts their activity. It is established that infection with the SARS-CoV-2 virus leads to fatal and severe cases of the disease, primarily among the elderly population. However, the underlying cause of the development of severe forms in elderly individuals remains incompletely understood. Thus, we hypothesized that the dysregulation of the Treg cell activity in elderly individuals may contribute to the development of severe forms and lethality from SARS-CoV-2, leading to the emergence of hyper inflammation in this population.

To investigate the impact of SARS-CoV-2 on the immunosuppressive properties of Treg cells, venous blood samples were obtained from young healthy volunteers and elderly individuals. Dendritic cells were obtained by PBMC adhesion on plastic. Adherent monocytes were cultured in cytokine medium with interleukin 4 (IL-4) and granulocyte-macrophage colony-stimulating factor (GM-SCF), with addition of lipopolysaccharide (LPS) after 48 hours and incubated for an additional 24 hours with S1 or N antigens of SARS-CoV-2 virus. The obtained dendritic cells presenting SARS-CoV-2 antigens were then co-cultured with autologous $CD4^+$-T lymphocytes *ex vivo*. The expression level of immunosuppressive membrane-associated and soluble molecules expressed by Treg cells was assessed using flow cytometry.

Our results indicate that the immunosuppressive activity of Treg cells exhibits contrasting patterns in the two donor groups studied in response to the presentation of SARS-CoV-2 antigens. The proportion of Treg cells, as well as the expression levels of the immunosuppressive molecules, including tumor growth factor beta (TGF-β), CD39, glucocorticoid-induced tumor necrosis factor receptor-related (GITR) protein, and the levels of the intracellular immunosuppressive cytokines, including IL-10 and IL-35, in Treg cells obtained from peripheral blood of elderly donors, significantly increased in response to the presentation of SARS-CoV-2 antigens *ex vivo*, when compared to the group of young donors. Conversely, in the group of young donors were observed a decrease in the expression of these markers and an increase in the concentration of extracellular pro-inflammatory cytokines IL-2 and IFN-γ *ex vivo*, which activate the effector immune cells and contribute to virus elimination.

In summary, the presented results suggest that Treg cells derived from elderly individuals exhibit elevated immunosuppressive activity in response to SARS-CoV-2 antigens *ex vivo.* Obtained results may indicate that in elderly individuals on the early stages of SARS-CoV-2 infection Treg cells may also possess enhanced immunosuppressive activity, which may contribute to the overall suppression of anti-viral immune responses, thereby potentially leading to the delayed SARS-CoV-2 virus clearance and development of severe forms of the disease.