Study of SARS-CoV-2 spike protein interaction with macrophages in presence of vitamin D

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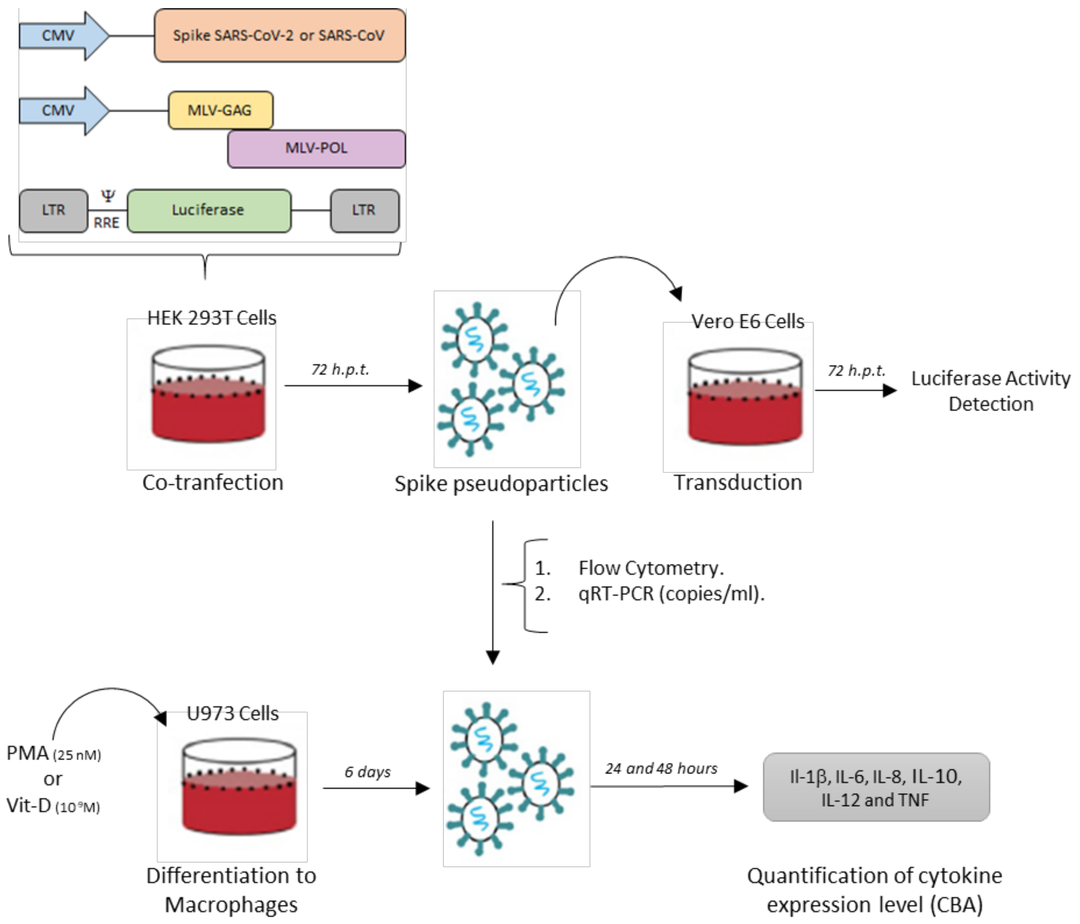
INTRODUCTION

The macrophages are one of the most important cell populations playing a role in the development of the inflammatory response in SARS-CoV-2 infection. Indeed, the altered function of macrophages in cases of severe COVID-19 leads to a hyperactivated inflammatory response possibly due to damage of the respiratory tract epithelium and/or the interaction with viral particles through the recognition of the Toll-like receptors (TLR), even in the absence of active viral replication. Moreover, the activation and function modification of macrophages could be exacerbated in conditions of vitamin D deficiency, as described dengue virus infection.

AIM

This study aims to evaluate the *in vitro* effect of the pseudovirions expressing the SARS-CoV or SARS-CoV-2 spike protein on the inflammatory cytokine response profile of macrophages grown in the presence or absence of vitamin D.

METHODS



RESULTS

The pseudovirions were obtained by co-transfection of three plasmids *i)* Gag-Pol of Murine Leukemia Virus (MLV), *ii)* Firefly luciferase gene/MLV RNA Ψ packaging signal/ 5'/3' flanking MLV LTR, and *iii)* SARS-2S Spike or SARS Spike (donated by Cornell University), in HEK-293 cells and harvested 72 hours post-transfection (h.p.t.).

The infectivity of the pseudovirions stocks was assessed in Vero E6 cells by transduction. Luciferase activity was determined 72 horas post-transduction (h.p.t.) and a title of 7,76 x 10^8 URL/ml was calculated based on the 10^{-5} dilution (Luciferases Assay System with Reporter Lysis Buffer kit, Promega).

The pseudotyped particles were characterized by flow cytometry, and indirect immunofluorescence using an SARS-CoV-2 Spike antibody, with a 13-color, 3-laser cytoFLEX S NVBR RVO flow cytometer (Beckman counter), operated with CytExpert v1.2 software. (Fig 1).

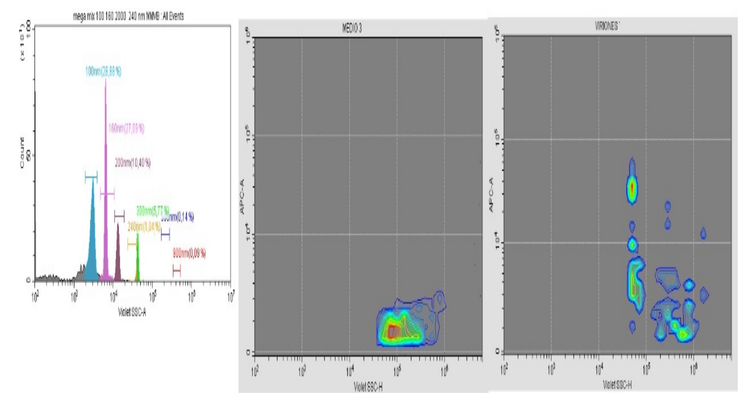


Figure 1. Characterization of pseudovirions by flow cytometry. Detection of particles of around 80 nm.

The stock of pseudovirions were also quantifed by Real Time PCR, previous RNA extraction and synthesis of cDNA using the primers for Luciferase described by Kong et al. 2018. The copy number of the plasmid Firefly luciferase gene/MLV Ψ RNA / 5'/3' MLV LTR was 288.631 copies/ml of SARS-CoV-2 pseudovirions stock (Fig 2).

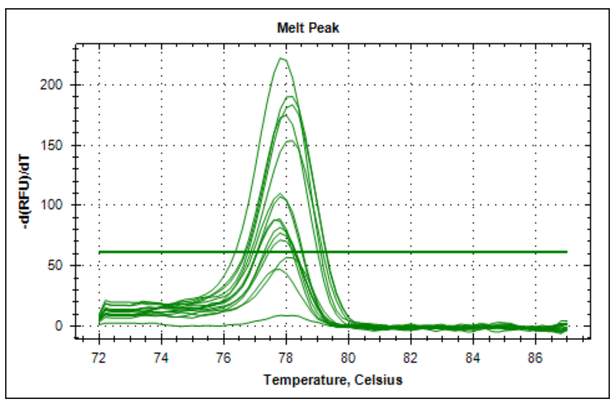


Figure 2. Quantification of copy number of the plasmid Firefly luciferase gene/MLV Ψ RNA / 5'/3' MLV LTR by Real Time PCR.

CONCLUSIONS

We are currently evaluating the expression of the cytokine profile in U973 cells, differentiated to macrophages with PMA and/or 1,25-dihydroxyvitamin D3, in presence of the pseudotyped particles expressing SARS-CoV-2 spike.

The results of this study may provide evidence to explain one of the mechanisms of COVID-19 pathogenesis.

ACKNOWLEDGMENT