Monocyte adherence to endothelial cells is facilitated by SARS-CoV-2 accessory protein ORF7A

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Introduction

Covid-19 is an ongoing pandemic with nearly 500 million cases worldwide caused by the SARS-CoV-2 virus. Covid-19 causes widespread inflammation and affects multiple organs and there has been speculation that it affects the endothelium, but the mechanism through which it does so is not known. The virus shares a wide similarity with SARS-CoV-1, where its spike protein uses the ACE2 receptor as a common pathway for entry into the cell. However, the accessory protein ORF7a also facilitates cell interactions due to its structural homology to ICAM-1, where it can bind to monocytes and T-cells via Mac-1 or LFA-1 respectively. We tested the hypothesis that SARS-CoV-2 ORF7a could facilitate increased adhesion of human monocytes isolated from peripheral blood mononuclear cells (PBMCs) to cultured endothelial cells (HUVECs).

Methods

The effect of recombinant ORF7a and an inflammatory cytokine (TNFα) were tested in vitro using a monocyte-endothelial cell adhesion assay. During the assay HUVECs were seeded in 96-plate wells, followed by a 24-hour exposure to treatments of either ORF7a, TNFα or a ORF7a and TNFα mix at varying concentrations. After 24 hours, CD14+ monocytes were tagged with calcein-AM and incubated with the cell monolayer for 2 hours. After fixing the monolayer, fluorescence was measured at excitation 485 nm and emission 515 nm.

Results

Monocyte adherence (as measured by calcein fluorescence after washing) increased by 50±10% when exposed to 1nM ORF7a ($p<0.05$ compared with control, N=3). This was comparable to that induced by 3nM TNFα . Likewise, cultures exposed to both ORF7a and TNFα mix experienced a dose dependent increase in monocyte adherence to 272±20% at 3.0nM concentrations (N=3) with an IC50 around 0.1nM.

Conclusions

These results indicate ORF7a's role in facilitating the binding of CD14+ monocytes to endothelial cells and enhancing the pro-inflammatory binding induced by TNFa stimulation. This could provide a mechanism through which SARS-CoV2 can cause inflammation that is independent of the Spike protein.

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