

Biologia vascolare

TOLL-LIKE RECEPTOR 4-MEDIATED ENDOTHELIAL DYSFUNCTION VIA NOX2 ACTIVATION IN COVID-19

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Background. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection is associated with endothelial dysfunction. Multiple pathophysiological mechanisms have been proposed, including direct endothelial infection and indirect injury mediated by systemic inflammation. However, alternative receptors involved in SARS-CoV-2–induced endothelial damage remain to be fully elucidated. We hypothesized that the SARS-CoV-2 Spike protein contributes to endothelial dysfunction via Toll-like receptor 4 (TLR4)-mediated oxidative stress.

Methods. We studied 20 patients with SARS-CoV-2 infection. Endothelial function was assessed by flow-mediated dilation (FMD). Serum levels of Spike protein and TNF-α were measured. In vitro, human umbilical vein endothelial cells (HUVECs) were incubated with recombinant Spike protein, TNF-α, or both, in the presence or absence of specific inhibitors of TLR4, NOX2, $p47^{phox}$, PKC, and ACE2. Markers of endothelial activation (NO bioavailability, endothelin-1) and oxidative stress (H_2O_2, sNOX2-dp, $p47^{phox}$ phosphorylation) were evaluated.

Results. Compared with controls, COVID-19 patients exhibited significantly reduced FMD. Linear regression analysis in the overall population showed that FMD inversely correlated with serum Spike protein and TNF-α levels. A significant positive correlation between TNF-α and Spike protein was also observed. In vitro, S protein (250 and 500 ng/mL) reduced nitric oxide (NO) bioavailability and significantly increased endothelin-1 levels compared with unstimulated HUVECs. These effects were attenuated by TLR4 or $p47^{phox}$ inhibition, whereas ACE2 inhibition (DX600) had no impact. Mechanistically, S protein increased H_2O_2 production, sNOX2-dp levels, $p47^{phox}$, and PKC phosphorylation in a dose-dependent manner. NOX2 activation and $p47^{phox}$ phosphorylation were reduced by TLR4 or $p47^{phox}$ inhibitors, while ACE2 inhibition did not affect S protein–induced oxidative stress. Exposure of HUVECs to TNF-α alone, at concentrations comparable to those observed in patients, or to S protein alone induced endothelial activation and oxidative stress. Co-incubation with TNF-α and S protein further amplified these effects, which were significantly reduced by inhibition of TLR4, S protein, TNF-α, or $p47^{phox.}$

Conclusions. The Spike protein directly induces endothelial oxidative stress and dysfunction through a TLR4–NOX2–$p47^{phox}$ signaling pathway, independently of ACE2. TNF-α synergistically enhances these effects. These findings identify TLR4-mediated signaling as a key mechanism underlying COVID-19–related vascular damage and suggest potential therapeutic targets to prevent thrombotic complications.