CD38 prevents IL-6-induced endothelial injury in SARS-CoV-2 infection

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PDF poster

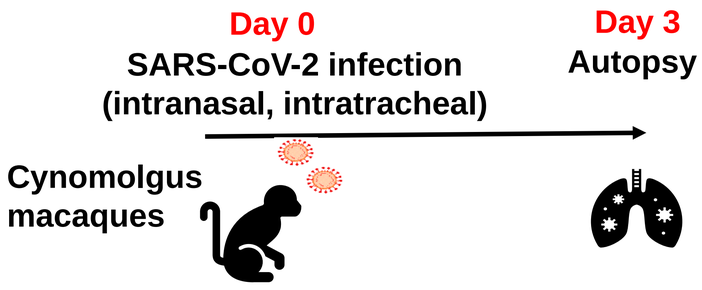
Abstract

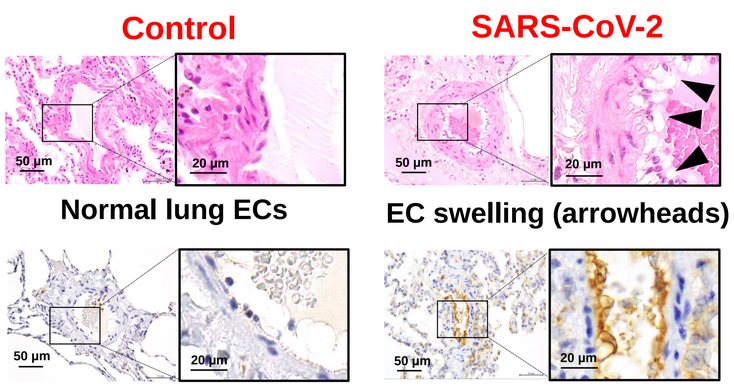
Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection has often been reported to induce vascular endothelial cell (EC) injury, which could activate platelets and the coagulation cascade, resulting in coagulopathy. Interleukin (IL)-6 has been shown to trigger EC injury since anti-IL-6 receptor antibody treatment effectively reduced SARS-CoV-2-induced EC injury. CD38 is a multifunctional molecule that can be upregulated on immune cells in inflammatory tissues. Previously, we found that EC injury was accompanied by CD38 expression on ECs in an experimental SARS-CoV-2 model, suggesting a role of CD38 in EC injury. In this study, we aimed to investigate whether CD38 could be associated with IL-6-induced EC injury. We activated human umbilical venous endothelial cells (HUVECs) with recombinant IL-6 (rIL-6); EC activation was confirmed by E-selectin upregulation. We found upregulations of CD38 and IL-6 in activated ECs, suggesting that CD38 and IL-6 could synergistically contribute to EC injury. Unexpectedly, however, CD38 depletion by an siRNA resulted in overactivation of ECs shown by higher levels of E-selectin and IL-6 at 6 hours, followed by EC apoptosis at 48 hours after rIL-6 treatment. Here, in the absence of CD38, IL-6 induced a cascade reaction of IL-6 production and EC activation, leading to EC death. Therefore, *in vitro,* CD38 could interfere with the positive feedback loop of IL-6 production/activation in ECs and protect ECs from IL-6-induced injury. In SARS-CoV-2 infection, other cell types can also produce/express IL-6 and CD38; interactions among these cells may determine the severity of EC injury.

Background

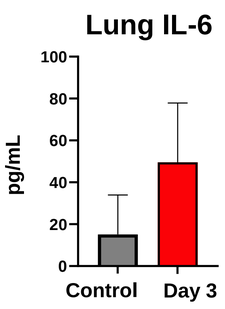
Endothelial cell (EC) activation and injury in SARS-CoV-2 infection

- CD38 is a multifunctional molecule that can be upregulated on immune cells in inflammatory tissues.
- Vascular EC activation with CD38 expression was observed in the infected macaques from day 3 post-infection.
- The role of CD38 expression in ECs during viral infection has not been clarified.

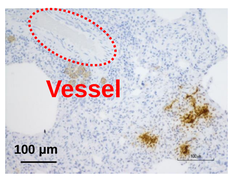




- Interleukin (IL)-6, but not virus, likely triggers EC injury (Kang et al, 2020).

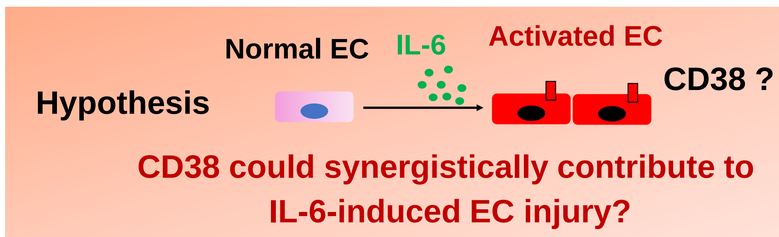


Viral antigens were found in interstitial tissues, but not in vascular ECs.

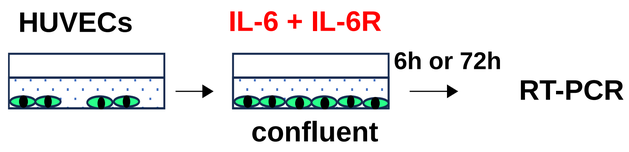


Increased IL-6 in SARS-CoV-2-infected macaque lungs.

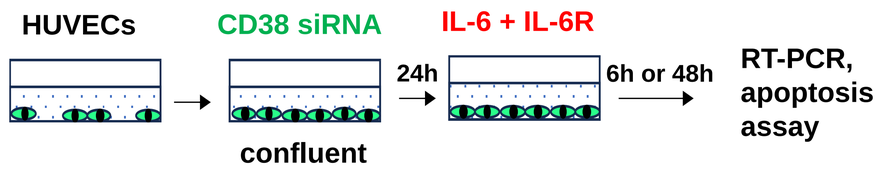
Viral antigens (brown)



Methods



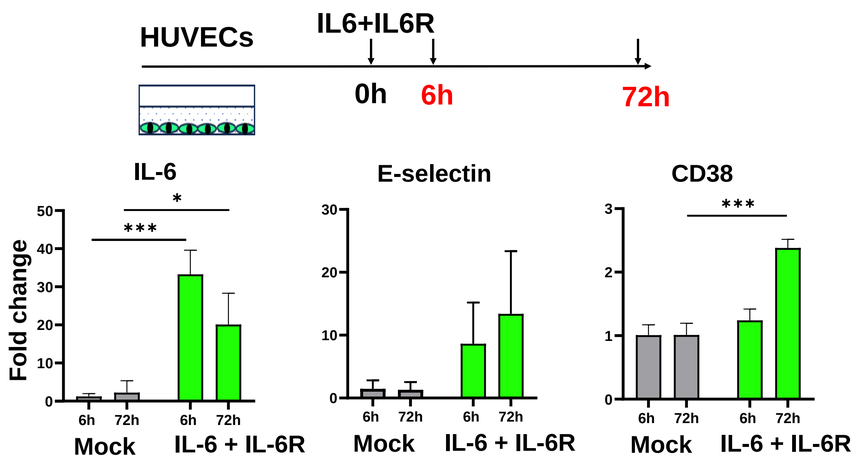
Human umbilical vein endothelial cells (HUVECs) were cultured in EGM-2™ Bulletkit™ medium in 24-well plates. The cells in confluence were stimulated with recombinant human IL-6 and IL-6 receptor (IL-6R) proteins (Kang et al, 2020).



HUVEC cells were treated with CD38 siRNA or control (-) siRNA using Lipofectamine™ RNAiMAX in Opti-MEM reduced serum medium before IL-6 + IL-6R stimulation.

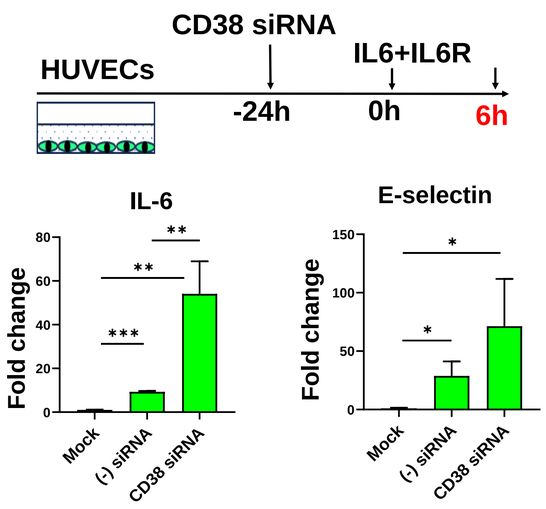
Results

IL-6 induces EC activation and CD38 upregulation



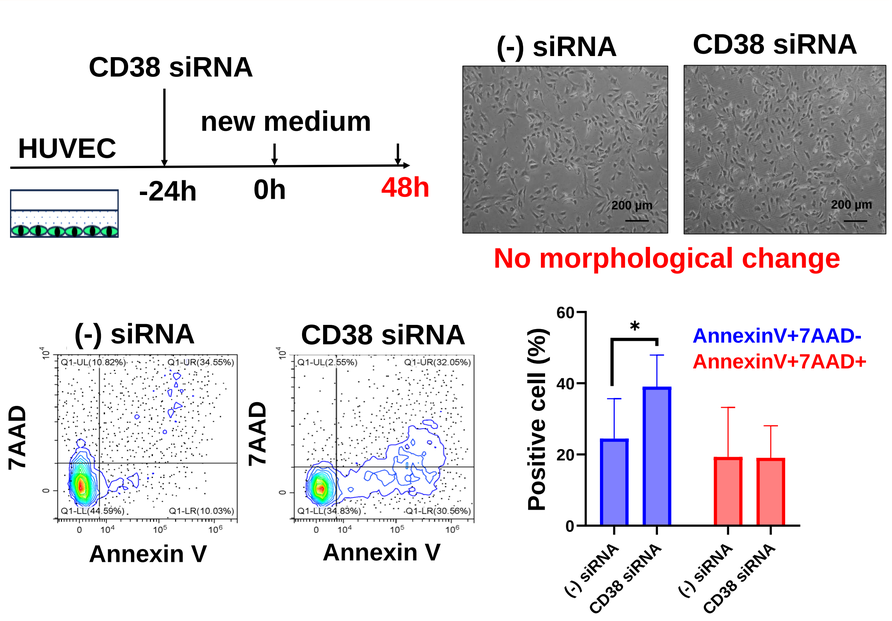
IL-6 + IL-6R induced significant upregulation of IL-6, E-selectin, and CD38. HUVECs were harvested at 6h and 72h for RT-PCR after being stimulated with IL-6 + IL-6R. Student's *t*-test: *, $p<0.05$; ***, $p< 0.001$.

Unexpectedly, CD38 depletion over-activates IL-6-stimulated ECs



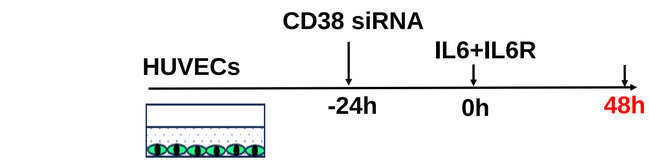
IL-6 + IL-6R significantly induced higher upregulation of IL-6 and E-selectin mRNA in CD38 mRNA-silenced cells than in the (-) siRNA and control cells. Cells were harvested at 6h for RT-PCR. ANOVA: *, $p<0.05$;**, $p<0.01$; ***, $p< 0.001$.

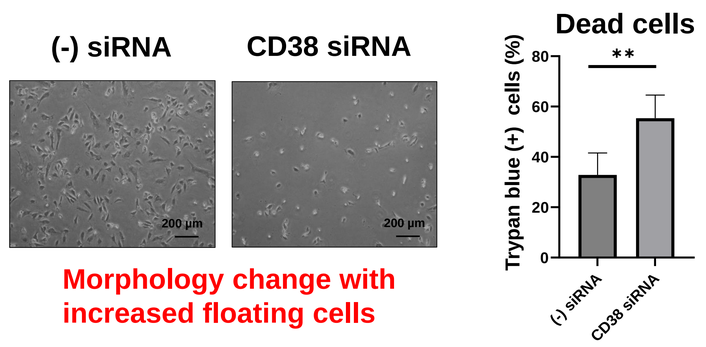
CD38 depletion alone induces EC apoptosis

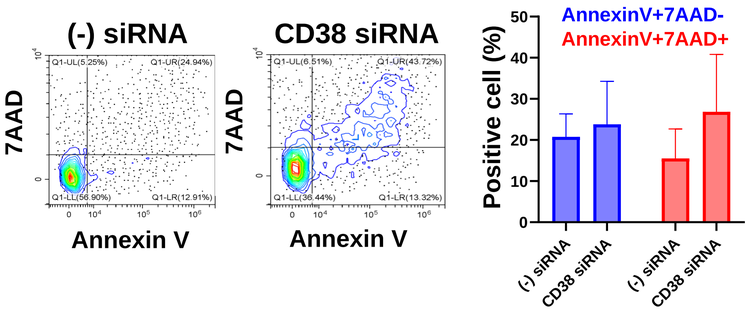


CD38 depletion alone induced early apoptosis of ECs with a significant increase of annexinV+7AAD- cells. HUVECs were stained with PE-annexin V and 7AAD, and acquired with a Beckmann Coulter CytoFLEX S flow cytometer. Student's *t*-test: **, $p<0.01$.

CD38 depletion induces EC death with IL-6 stimulation



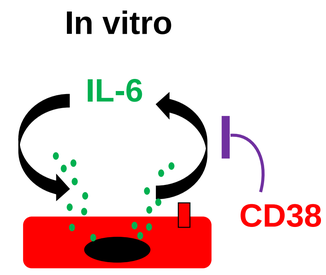




CD38 depletion with IL-6 stimulation induced cell death with increased late apoptosis or necrosis (annexinV+7AAD+ increase). The dead cells were detected with trypan blue using a Bio-rad TC20™ Automated Cell Counter.

Discussion

- During SARS-CoV-2 infection, IL-6 could activate ECs with CD38 upregulation. CD38 is an enzyme degrading nicotinamide adenine dinucleotide (NAD), suggesting that NAD dysmetabolism could occur in ECs.
- In viral infections, both beneficial and detrimental roles of CD38 have been reported (Boslett et al., 2018; Lischke et al., 2013; (Di Pierro et al., 2022).
- Our results showed that in vitro, CD38 could inhibit the positive feedback loop of IL-6 and protect ECs from IL-6-induced injury.



- In vivo, interactions among the cells that produce or express IL-6/CD38 may determine the severity of endothelial injury and SARS-CoV-2 infection.

Conclusions

- IL-6 activates endothelial cells with CD38 expression.
- Unexpectedly, CD38 depletion by siRNA results in endothelial overactivation and cell death after IL-6 stimulation.
- CD38 likely protects ECs by inhibiting the positive feedback loop of IL-6.

Reference, Grant, and COI

- Nguyen, C.T., Itoh, Y., Tsunoda, I. Potential roles of CD38 and HIF for vascular endothelial dysfunction in SARS-CoV-2 and Theiler's virus infections. *Medical Science Digest*, Vol 49 (7), 388-390, 2023.

- Supported by KAKENHI No. 21K20757

- I have no COI.