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INVESTIGATION OF THE INTERACTION BETWEEN SPIKE PROTEINS OF DIFFERENT SARS-COV-2 VARIANTS AND THE ACE2 RECEPTOR

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Initial membrane-coupled events of SARS-CoV-2 infection involve interactions between viral spike proteins and lipid raft resident ACE2 and TMPRSS2 proteins of the host cell. We found that remdesivir formulations containing cyclodextrins (CDs) inhibit ACE2 binding and internalization of spike proteins due to membrane cholesterol depletion and raft disruption independent of remdesivir, raising the therapeutic applicability of CDs in COVID-19.

We hypothesized that SARS-CoV-2 variants with different ACE2 affinities are characterized by different cholesterol and thus CD sensitivity. Our aim was to compare ACE2 binding affinities of spike receptor-binding domains (RBDs) of clinically relevant variants (WT, Alpha, Beta, Delta, Omicron Ba1 and Ba2), and examine the relationship between their binding affinity, cholesterol sensitivity and lipid raft preference. We are also investigating the effects of cholesterol extraction on the uptake of spike trimer variants.

In HEK293 cells stably expressing ACE2 and TMPRSS2, using flow cytometry we found that spike RBD variants exhibited different binding efficiency to ACE2, and a negative correlation was observed between binding efficiency and the extent of hydroxypropyl- β -cyclodextrin (HP β CD)-mediated inhibition of ACE2 binding. We also examined the correlation between receptor binding and cellular lipid raft content quantified using either fluorescently labeled GM1 ganglioside-binding cholera toxin B subunit or an environment-sensitive fluorophore (F66). We found that variants with lower ACE2 affinity (WT or Omicron Ba1) showed stronger preference for cells with higher lipid raft abundance than those with higher affinity (Delta). Using confocal microscopy and quantitative 3D image analysis, we are currently investigating if these changes are mirrored in an altered efficiency and HP β CD sensitivity of cellular uptake of different trimer variants.

SARS-CoV-2 spike protein variants are characterized by distinctive ACE2 binding efficiency and cholesterol sensitivity, which could originate from their altered preference to raft-resident ACE2 over receptors found in non-raft membrane regions. Consequently, receptor binding and internalization of the high affinity variant Delta exhibits lower cholesterol sensitivity compared to that of the lower affinity variant Omicron Ba1.

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