Cellular Responses to Treatment with Spike Protein from Omicron (B.1.1.529) and Alpha (B.1.1.7) Variants of SARS-CoV-2.

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Introduction.

According to previous research, the Interleukin-6 Receptor (IL-6R) gene's rs2228145 variant may influence patients' responses to COVID-19 infection[1]. Using monocytic cells that express different forms ('genotypes') of this variant: U937 [AA], THP-1 [AC] and MM6 [CC][2], we investigated the impacts both of this variant on IL-6 Trans-signaling responses to SARS-CoV-2 Spike Protein (SP) treatment, and of blocking agents on such responses. **Our aims were**:

[i] to determine whether responses to SP of the Alpha coronavirus strain (Alpha-SP) differ from responses to SP of the Omicron coronavirus strain (Omicron-SP);

[ii] to elucidate the impact of the IL-6:IL-6R blocker drug Tocilizumab on SP-evoked IL-6 trans-signaling.

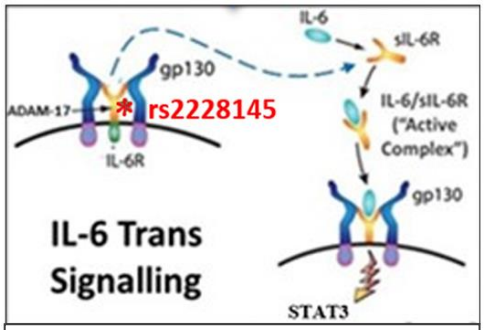


Fig 1: rs2228145 impacts on IL-6 Trans-signaling. A→C change at IL-6R gene position 1073 (* on Fig) leads to Asp→Ala[358] amino acid change in IL-6R protein, greater sIL-6R shedding and enhanced IL-6 Trans signaling.

Results.

[i] Cells of CC genotype produced the most IL-6:sIL-6R 'active complex' *(~8pM)*, while AA cells produced the least *(~5.8pM).* [ii] Both SPs raised 'active complex' levels but Alpha-SP generally evoked larger increases than Omicron-SP. [iii] Alpha-SP also triggered STAT3 phosphorylation *(a downstream step in IL-6 Trans-signaling),* but this was inhibited by pre-incubation with Tocilizumab before SP treatment.

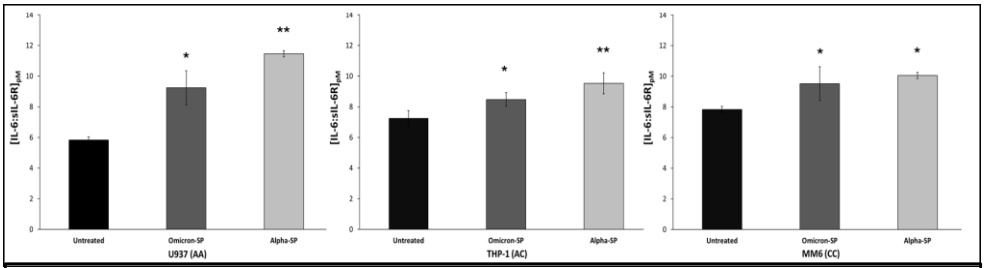


Fig 2: Alpha-SP or Omicron-SP raise active complex levels in U937, THP-1, MM6 cells; Alpha-SP evokes larger responses than Omicron-SP. *(Alpha-SP or Omicron-SP [1nM,24h] as indicated; *p<0.05 v control; **p<0.05 v Omicron-SP).*

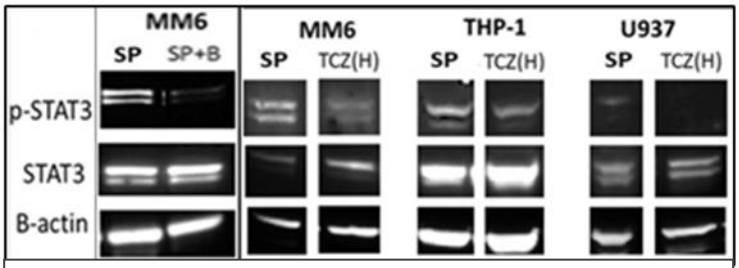
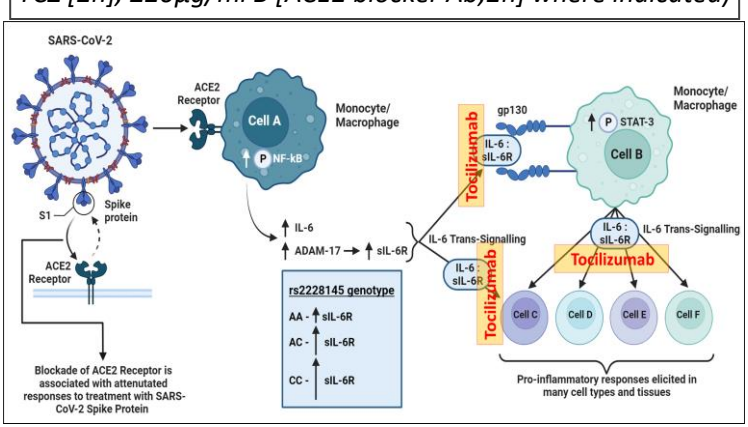


Fig 3: Tocilizumab (TCZ) inhibits Alpha-SP-induced ACE2-dependent STAT3 phosphorylation (pSTAT3:STAT3 ratio) in U937, THP-1, MM6 cells. *(1nM Alpha-SP [24h]; ±50ng/ml TCZ [2h], ±20μg/ml B [ACE2 blocker Ab;2h] where indicated)*

Discussion/Conclusions:

- Increased sIL-6R shedding exacerbates pro-inflammatory IL-6 Trans-signaling in CC cells. ***(Does this also occur in CC COVID-19 patients?)***
- While both SP-variants triggered IL-6 trans-signaling, Alpha-SP responses were generally significantly stronger than those of Omicron-SP. ***(Could reported differences in SARS-CoV-2 strains' pathogenicity [see [3]] stem from Alpha-SP's greater ability than Omicron-SP to increase IL-6:sIL-6R production?)***
- Tocilizumab blocking of downstream STAT3 phosphorylation suggests potential for this drug in treating COVID-19 or long-COVID. ***(Could Tocilizumab be more effective in CC patients?)***



References: **[1]** Bovjin et al 2020. Lancet. 2(11), pp. e658–e659. doi: 10.1016/S2665-9913(20)30345-3. **[2]** Sarwar et al 2023. COVID 3(10), pp. 1554–1570. doi: 10.3390/covid3100106. **[3]** Andre, et al. 2023. Biology 12(9), p. 1267. doi: 10.3390/biology12091267.