Activation of Macrophages Enhances Susceptibility to SARS-CoV-2 Antibody-Dependent Enhancement and Promotes Damage to Downstream Epithelial Cells

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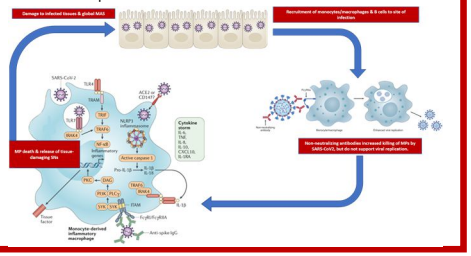
Background & Significance

- The distinct shift in peripheral monocyte activation and infiltration of these cells into the respiratory tract observed in severe cases of COVID-19 suggests that like SARS-CoV, the acute respiratory distress syndrome (ARDs) observed in SARS-CoV-2 infections may result from damage to the respiratory epithelia by improperly activated macrophages (MPs).
- In this study, we examined the ability of non-neutralizing antibodies to sensitize MPs to killing by SARS-CoV-2, as well as the impact of these cells on downstream epithelial cells.



Methods

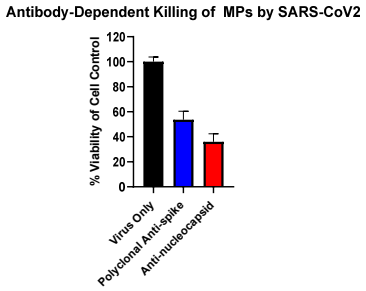
Viability and Infection Assays

Raw 264.7 cells were seeded into 96-well plates at a density of 1x10^4/well and incubated overnight in the presence or absence of heat-inactivated LPS derived from either *E. coli* (EC) or *S. enteritidis* (Sal). Cells were then treated with non-neutralizing antibodies or vehicle control at the time of infection with SARS-CoV-2. Viability was assessed 48 hours post-infection by luminescence following the addition of CellTiter-Glo® (Promega).

Assessment of Viral Replication by qPCR

Raw 264.7 cells were seeded at a density of 1x106 cells/well in a 12-well plate and allowed to settle overnight. The following day, cells were infected with SARS-CoV2 with an MOI of 0.05 and incubated overnight at 37C with 5% CO2. Cells were lysed with Trizol and RNA extracted using a Direct-zol™-96 MagBead RNA miniprep kit (Zymo). Quantitative PCR was performed using the VIRSeek SARS-CoV2 assay (Eurofins Genescan Technologies, Freiberg, Germany).

Results



Killing of MPs is Independent of Viral Replication

Sample ID	Average Ct
Naïve no virus	ND
Naïve+virus	26.6±0.2
Naïve+Ab+virus	28.4±1.85
LPS+virus	27.27±0.9
LPS+Ab+virus	28±0.2

Figure 1 and Table 1. Exposure to SARS-CoV2 alone had little impact on Raw264.7 cells (n=17). The presence of non-neutralizing antibodies at a concentration of 6.25 ug/ml, SARS-CoV2 significantly reduced MP viability to 35.98% and 53.67%, respectively (p<0.0001 and p=0.0003, n=14). Surprisingly, despite significant cell death, no increase in viral replication was observed in any of the macrophage samples suggesting that killing of MPs by SARS-CoV2 iccurs thru an as yet unknown mechanism. Independent of viral replication.

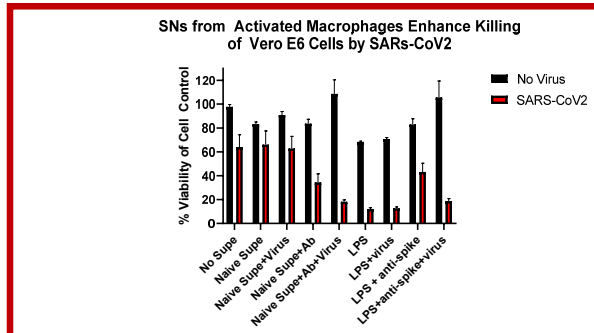


Figure 3. Supernatants collected from naïve MPs subjected to ADE markedly increased the susceptibility of Vero e6 cells to SARS-CoV2 nearly 9.8-fold (p<0.0001, n=12). Similarly, supernatants from MPs sensitized to SARS-CoV2 thru activation with EC LPS and Sal LPS also increased viral killing of Vero e6, reducing cell viability to 13.03% (p<0.0022, n=8).

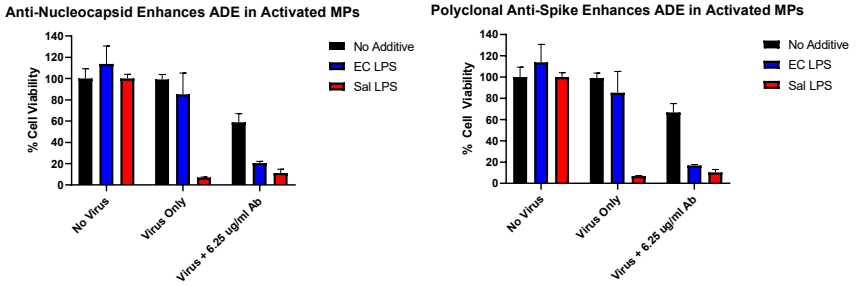


Figure 2. Activation of MPs with Sal-derived LPS sensitized MPs to viral killing, even in the absence of non-neutralizing antibody (20.12% viability, p<0.0001, n=18). MP activation by both Sal and EC LPS further enhanced viral killing in the presence of anti-nucleocapsid, reducing cell viability to 12.21% (0.0001, n=18) and 6.46% (p<0.0001, n=18).

Discussion

- Macrophages are resistant to killing by SARS-CoV2 in the absence of non-neutralizing antibodies
- Antibodies that do not neutralize the RBD region of the spike protein renders macrophages susceptible to the virus
- Killing of MPs by SARS-CoV2 does not result from viral replication
- Activation of macrophages thru TLRs also enhances sensitivity to SARS-CoV2, suggesting that the underlying state of inflammation may also play a role in determining the severity of COVID-19
- MPs secrete as yet unknown factors that enhance susceptibility of VeroE6 cells to SARS-CoV2, providing a route thru which components from virus-activated MPs directly damage surrounding respiratory tissues in the later stages of SARS-CoV2 infection.

- Taken together, this suggests that the MAS-like syndrome observed in severe COVID-19 may result from improper processing of the virus in the presence of non-neutralizing antibodies