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SARS-CoV-2 cellular tropism

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In *The Lancet Microbe*, Hin Chu and colleagues[1] compare the replication capability of severe acute respiratory syndrome coronavirus (SARS-CoV) and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in 25 different cell lines, including nine of human origin, using quantitative RT-PCR. The susceptibility of different cell lines to SARS-CoV and SARS-CoV-2 offers valuable information for future work on antiviral compounds, vaccine strain production, investigation of cellular interactors, and host responses to infection. Moreover, the results obtained in the permissive animal cells might be an indicator of angiotensin-converting enzyme (ACE) 2 cross-species recognition.

Chu and colleagues identified several cell lines in which both SARS-CoV and SARS-CoV-2 replicated efficiently, but cytopathic effects were only seen in the non-human primate kidney cell lines VeroE6 and FRhK4, including cell rounding, detachment, and degeneration. VeroE6 is the standard cell line used to produce viral stocks of SARS-CoV-2 in different laboratories and to do plaque assays.[2] The noted cytopathic effects are consistent with use of this cell line in virology because of the susceptibility of VeroE6 cells to infection, lower saturation density (optimal for plaque formation), and interferon α and interferon β secretion deficiency.[2] The FRhK4 cell line has been studied less than has VeroE6 and, hence, further investigations to enlighten the cytopathic effects in the context of an intact innate immune response will be relevant.

Chu and colleagues analysed several other animal epithelial kidney cell lines.[1] A significant increase of viral load between 2 h postinoculation (hpi) and 120 hpi was seen in LLCMK2 (monkey), RK-13 (rabbit), PK-15 (pig), and CRFK (cat) cell lines. These results do not fully recapitulate the animal tropism reported so far: findings of studies of SARS-CoV-2[3] and SARS-CoV have highlighted possible infections in cats (and felines in general), ferrets, and hamsters, whereas rabbits were not investigated, dogs seemed (until now) to be poorly permissive, and pigs were not infectable. These discrepancies might derive from the kidney origin of the cells, their immortalisation, or the culturing conditions.

Different bat cell lines were also assessed by Chu and colleagues, including in *Rhinolophus sinicus* kidney (RSK) and lung (RSL) cells.[1] Only SARS-CoV showed significant growth in RSK cell lines, although at reduced extent compared with other animal cell lines, and no replication was evident in RSL cell lines. Further studies will need to investigate the susceptibility of cell lines from *Rhinolophus affinis*, the bat species in which the sequence of the bat coronavirus RaTG13 (the virus phylogenetically closest to SARS-CoV-2) was identified.[4] Moreover, in bats, coronaviruses are often detected in anal swabs; therefore, animal gastrointestinal epithelial cell lines should be assessed in future studies.

Chu and colleague also compared various human cell lines, including pulmonary (Calu3), intestinal (Caco2), hepatic (Huh7), and neuronal (U251) cells. However, some anatomical sites or cell types (eg, cardiomyocytes) were not represented or only one cell line was used to represent a site; for example, with use of only U251 cells, the presented findings are not sufficient to draw conclusions on neuroinvasiveness, particularly since they contrast with previous research.[4] SARS-CoV-2 replication was greatest in Calu3 and Caco2 cell lines, and these anatomical sites (pulmonary and intestinal) are the ones positive for viral RNA in humans.[5]

Notably, SARS-CoV-2 grew in Calu3 cells, but not in A549 cells, although both cell lines derive from lung adenocarcinoma. This finding accords with previous results in A549 cells, which were infectable only on overexpression of ACE2,[6] whereas use of Calu3 cells has been reported elsewhere.[7] SARS-CoV-2 was shown to grow faster and at a higher titre than SARS-CoV in Calu3 cells, in agreement with findings of earlier work by Chu and colleagues,[8] in which the susceptibility of ex-vivo human lung tissues to both SARS-CoV-2 and SARS-CoV was assessed. SARS-CoV-2, despite the higher titre, does not induce interferon secretion and causes lower upregulation of proinflammatory cytokines than does SARS-CoV.[8] These results suggest a better immune evasion of SARS-CoV-2, but a more mechanistic investigation is needed to draw conclusions.

Conversely, SARS-CoV was shown to replicate better than SARS-CoV-2 in Caco2 cell lines. The descriptive data obtained accord with the symptoms of patients with coronavirus disease 2019 (COVID-19); diarrhoea was reported in 3·7% of cases of COVID-19 in China,[9] whereas up to 38·4% of patients with severe acute respiratory syndrome reported diarrhoea.[10] Future research should

be done in primary cell lines, human derived organoids, or pseudostratified epithelia, which more faithfully mimic the physiological structure and cell differentiation of the human intestine. Similar to the analysis done in ex-vivo lungs,[8] immune activation should be assessed.

In conclusion, the study by Chu and colleagues offers possible directions for designing more in-depth studies about animal and human susceptibility to SARS-CoV-2 infection. However, we should keep in mind that results obtained in cell lines might not always recapitulate the effects in the whole organism.

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