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SARS-CoV-2 and the safety margins of cell-based biological medicinal products

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ABSTRACT

With the pandemic emergence of SARS-CoV-2, the exposure of cell substrates used for manufacturing of medicines has become a possibility. Cell lines used in biomanufacturing were thus evaluated for their SARS-CoV-2 susceptibility, and the detection of SARS-CoV-2 in culture supernatants by routine adventitious virus testing of fermenter harvest tested.

1. Introduction

A general concern in cell-based manufacturing of recombinant proteins, including vaccines, is the potential contamination of the cell culture with viruses, which has had severe consequences for patients and manufacturers [1,2]. With the pandemic emergence of SARS-CoV-2, the exposure of biomanufacturing cell lines to a new virus has become a possibility, and to safeguard biomedicines it is important to understand whether the virus is even capable of infecting bioproduction cell lines, and if so, whether the event would be detected by adventitious agent tests (AAT) that are part of biomanufacturing quality control.

This study investigated four widely used biomanufacturing cell lines, i.e. CHO and Vero, as well as the two human cell lines HEK-293 and HT-1080, for their susceptibility to infection with SARS-CoV-2.

In addition, adventitious virus testing (AAT) as routinely performed for in-process control testing per regulatory guidance [3,4], was evaluated for its ability to detect SARS-CoV-2 infection if it were to occur. As MRC-5 cells are part of the AAT detector cell line panel (Fig. 1), these cells were also tested for their susceptibility to SARS-CoV-2.

2. Materials and methods

2.1. Virus and cells

SARS-CoV-2 strain BetaCoV/Germany/BavPat1/2020 (kindly provided by Charité Universitätsmedizin, Institute of Virology, Berlin, Germany; EVAg 026V-03883) was used for all inoculations. Any work with SARS-CoV-2 was done under biosafety level 3 (BSL-3) containment conditions.

CHO cells (Chinese hamster ovary, ATCC CCL-61), Vero cells (African green monkey kidney epithelial, CCL-81, sourced from the ECACC 84113001), HEK-293 cells (Human embryonic kidney cell line, ATCC CRL-1573), HT-1080 cells (Human fibrosarcoma cell line, ATCC CCL-121), and MRC-5 cells (human lung fibroblast, ATCC CCL-171) were used.

2.2. Inoculation of cells with SARS-CoV-2 and adventitious agent testing

Cells (CHO, Vero, HEK-293, HT-1080 and MRC-5) in 6-well plates

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were incubated with virus for 14 days. Inoculations with virus were done at multiplicity of infection (MOI) = 0.01 and MOI = 1, that is, approx. 10^3 and 10^5 infectious virus particles per well, respectively, for 1 h. Then the respective medium was added to a total volume of 6 ml/well (CHO medium: Ham's F12 medium supplemented with 10% FCS [PAN-Biotech P40-2009], L-glutamine [2 mM], nonessential amino acids [1x], sodium pyruvate [1 mM] and Gentamycin sulfate [100 mg/ml]; Vero medium: TC-Vero medium supplemented with 5% FCS, L-glutamine [2 mM], nonessential amino acids [1x], sodium pyruvate [1 mM], Gentamycin sulfate [100 mg/ml] and sodium bicarbonate [7.5%]; HEK 293 medium: EAGLE-MEM (+Earles BSS) supplemented with 5% FCS and Gentamycin sulfate [100 mg/ml]; HT-1080 medium: McCoy 5A supplemented with 2% FCS, L-glutamine [2 mM], sodium pyruvate [1 mM] and Gentamycin sulfate [100 mg/ml]; MRC-5 medium: EAGLE-MEM (+Earles BSS) supplemented with 10% FCS, L-glutamine [2 mM], nonessential amino acids [1x], sodium pyruvate [1 mM], Gentamycin sulfate [100 mg/ml] and sodium bicarbonate [7.5%]). 6-Well plates were incubated for 14 days at 36 °C and 4.5% CO_2. The presence of CPE was assessed on days 2, 3, 6, 7 and 14 post-infection (p.i.).

The presence of infectious SARS-CoV-2 was tested by titration at various stages throughout the incubation period, on Vero cells using a $TCID_{50}$ assay (see below). Samples for titration were taken from the input inoculum (day 0 p.i.) and days 2 (Vero cells MOI = 1 only), 3, 6, 7 and 14 p.i.

For each cell line, inoculations with SARS-CoV-2 at the two MOIs was done in duplicate.

In addition to SARS-CoV-2 readout by CPE, after 14 days of inoculation, hemadsorption (HAD) and hemagglutination (HA) assays were done, as routinely performed during adventitious agent testing. Due to the CPE detected, Vero cells were evaluated for HAD and HA on day 3 of incubation.

For HAD, wells with MRC-5, Vero, CHO, HEK-293 and HT-1080 cells were covered with erythrocyte suspensions of two different species (human 0.5% [v/v] and guinea pig 1% [v/v]) and incubated at 4 °C for 30 min. The cell culture supernatant was then removed, and the cells washed twice with PBS before microscopic inspection for HAD.

For HA, supernatants of MRC-5, Vero, CHO, HEK-293 and HT-1080 cells were diluted with 0.9% [w/v] NaCl solution in twofold steps. Erythrocyte suspensions (human, 0.5% [v/v] and guinea pig 1.0% [v/v]) were added and separate V-shaped plates incubated for 35 min at 4 °C and 36 °C, before hemagglutination was inspected visually. The described AAT is essentially the routine procedure used for the testing of recombinant protein bulk harvests.

2.3. Infectivity assay ($TCID_{50}$)

Samples that potentially contained infectious virus were titrated by tissue culture infectious dose 50% ($TCID_{50}$) assay, that is, eightfold replicates of serial half-log sample dilutions were incubated with cells for 5–7 days and CPE was assessed microscopically. Virus concentrations were calculated according to the Poisson distribution and expressed as viral load (log_{10} [$TCID_{50}$]).

3. Results and discussion

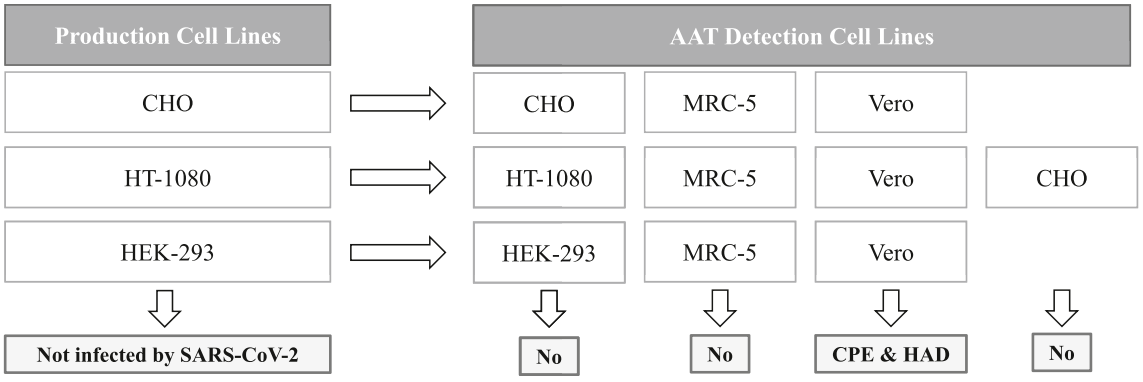
Five cell lines (CHO, Vero, HEK-293, HT-1080 and MRC-5) were inoculated in duplicate with SARS-CoV-2 at two different multiplicity of infection (MOI) values of 1 and 0.01, respectively, and incubated for 14 days. During the incubation period the cells were periodically observed for cytopathological effects (CPE), as an indicator for viral infection in the same way as performed during routine AAT.

No CPE was detected throughout the incubation period for CHO cells, HEK-293 cells, HT-1080 cells and MRC-5 cells. In contrast, Vero cells displayed a CPE already on day 2 (MOI = 1) or day 3 (MOI = 0.01) post infection (p.i.). The permissiveness of Vero E6 and CCL81 cells for SARS-CoV-2 infection and production of CPE has already been demonstrated [5], and thus Vero CCL81 cells were used in this study as positive control, as well as for quantitation of SARS-CoV-2 replication in the other cell lines evaluated: the presence of infectious virus in the cell culture supernatant was determined at regular intervals throughout the incubation period by tissue culture infectious dose 50% ($TCID_{50}$) assay, starting with the inoculum preparations and up to the last day of incubation (day 14).

For Vero cells inoculated with MOIs of 1 and 0.01, this analysis showed increasing SARS-CoV-2 titers in cell supernatants from day 2 and day 3 p.i., which peaked on days 6 and 7 p.i., at mean titers of 6.1 and 5.7 log_{10} $TCID_{50}$/mL, and after 14 days p.i. slightly dropped by on average of 0.7 and 0.8 log_{10}, respectively (Table 1).

For the CHO, HT-1080 and MRC-5 cell lines, no SARS-CoV-2 infectivity was detected by titration on Vero cells throughout the entire incubation period, which indicates that SARS-CoV-2 cannot replicate in these cells.

For HEK-293 cells inoculated at MOI = 0.01, SARS-CoV-2 infectivity was never detectable in culture supernatants. After inoculation at MOI = 1, however, low and constantly declining SARS-CoV-2 infectivity titers were detected until day 7 p.i. (Table 1). Whether these declining levels of virus reflect residual inoculum which may be more effectively bound by the HEK-293 as opposed to the CHO, HT-1080 and MRC-5 cell layers, or even some limited yet abortive replication as observed for other HEK-293-derived cells [5] remains to be determined.



CPE, cytopathological effects; HAD, hemadsorption.

Fig. 1. Overview of production cell lines evaluated for SARS-CoV-2 susceptibility and the corresponding detection cell lines that make up the respective AAT panels. The information in boxes below indicate whether SARS-CoV-2 permissiveness was detected and for Vero cells the successful assay readouts.

Table 1
SARS-CoV-2 titers ($TCID_{50}$/ml) in supernatants of Vero and HEK-293 inoculated in duplicate at MOIs of 0.01 and 1, respectively. The value of 1.1 $TCID_{50}$/ml represents the detection limit of the assay.

Cell line (MOI)	Days post infection					
	0	2	3	6	7	14
Vero (0.01)	2.0	NT	4.9/4.9	5.4/5.6	5.6/5.8	4.7/5.0
Vero (1)	4.1	5.1/ 5.2	5.1/5.4	6.1/6.1	6.0/5.9	5.4/5.3
HEK-293 (0.01)	2.4	NT	≤1.1/ ≤1.1	≤1.1/ ≤1.1	≤1.1/ ≤1.1	≤1.1/ ≤1.1
HEK-293 (1)	4.5	NT	3.2/3.1	2.2/2.5	1.4/≤1.1	≤1.1/ ≤1.1

MOI, Multiplicity of infection; NT, not tested.

In addition to the monitoring for the development of any CPE, the cells and supernatants were assayed at the end of the 14 days incubation period in the two standard AAT readouts, i.e., hemadsorption (HAD) to the cells, and hemagglutination (HA) activity in the supernatant, using human and guinea pig erythrocytes. This panel of altogether three assay readouts (CPE, HAD, HA) reflects exactly what is routinely performed during quality control testing of biomanufacturing fermenter harvests by using at least three different indicator cell lines (Fig. 1). The choice of detection cells lines for AAT includes per FDA guidance (i) the type of cells used for biopharmaceutical production, (ii) a human diploid cell line, e.g. MRC-5 cells, and (iii) a monkey kidney cell line, e.g. Vero cells [3].

For SARS-CoV-2 inoculated CHO cells, HT-1080 cells, HEK-293 cells and MRC-5 cells, virus was not detectable by any of the three AAT readouts, i.e. CPE as monitored throughout the two weeks of incubation and HAD/HA at the end of the incubation period. For Vero cells though, SARS-CoV-2 replication was, in addition to detection by CPE, detected by HAD with human erythrocytes (MOI = 1 only). These findings confirm that AAT would confidently detect a SARS-CoV-2 contamination in a biomanufacturing cell culture tested, so long as Vero cells are included as a detector cell line.

Entry of SARS-CoV-2 into susceptible cells is facilitated by the same receptor as for SARS-CoV, i.e. the Angiotensin-converting enzyme-2 (ACE 2) [6,7], which suggests that the spectrum of susceptible cells is similar for SARS-CoV and SARS-CoV-2. High abundance of ACE2 as a cellular surface protein on Vero cells [8] does correlate well with their susceptibility to infection with SARS-CoV-2. In contrast, the expression of ACE2 at low levels on CHO cells [9] might be the reason that SARS-CoV-2 replication in these cells was not observed. HEK-293 cells carry ACE2 at higher levels [9], which may facilitate binding and possibly entry of SARS-CoV-2 into HEK-293, followed by minimal and ultimately abortive replication (Table 1), or modest replication in HEK-293T cells [5]. However, the presence of a specific receptor may be the reason for virus entry in a target cell line but not the only requirement for productive virus replication. For SARS-CoV-2, 332 different protein-protein interactions have been identified [10], each providing for an interaction where virus proliferation might be blocked.

4. Conclusions

Permissiveness for proliferation of SARS-CoV-2 was confirmed for Vero cells, but was absent for CHO, HT-1080 and MRC-5 cells. HEK-293 cells did not show productive amplification of SARS-CoV-2 although some minimal and self-limiting replication may be possible. This study also showed that in CHO cells, HT-1080 cells and MRC-5 cells, no "silent" infection of SARS-CoV-2 did occur, i.e. virus replication but no development of detectable CPE, as a "silent" virus replication would have been detected by the titration of supernatants on Vero cells. In routine AAT assays, SARS-CoV-2 was confidently detected by CPE in Vero cells and HAD with human erythrocytes, i.e. two different readouts that revealed the presence of SARS-CoV-2, which underlines its reliable detection by AAT if it were to occur as a contaminant in a biomanufacturing cell culture process.

The detection of SARS-CoV-2 in AAT when Vero cells are part of the indicator cell line panel was reassuring, even in the hypothetical case that SARS-CoV-2 would replicate in the production cell line but would not produce a CPE and not be detected by cell parameter screening, for example by measurement of cell viability, oxygen consumption etc.

Furthermore, SARS-CoV-2 would represent a contaminant for which highly effective clearance capacities can be expected from widely used dedicated down-stream virus reduction steps: for example, as a lipid-enveloped virus SARS-CoV-2 would be robustly inactivated by solvent/detergent (S/D) treatment [11] and due to its large size of about 120 nm SARS-CoV-2 can be expected to be completely retained by nanofiltration, even when using larger pore sizes such as 35 nm [12].

Declaration of competing interest

J.M., A.K., M.R.F., and T.R.K are employees of Baxter AG, Vienna, Austria, now part of the Takeda group of companies. J.M., M.R.F. and T.R.K. are Takeda stock owners.

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