

SHORT COMMUNICATION

Severe acute respiratory syndrome coronavirus 2 and influenza A virus co-infection alters viral tropism and haematological composition in Syrian hamsters

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Abstract

The ongoing coronavirus disease 2019 pandemic and its overlap with the influenza season lead to concerns over severe disease caused by the influenza virus and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) co-infections. Using a Syrian hamster co-infection model with SARS-CoV-2 and the pandemic influenza virus A/California/04/2009 (H1N1), we found (a) more severe disease in co-infected animals, compared to those infected with influenza virus alone but not SARS-CoV-2 infection alone; (b) altered haematological changes in only co-infected animals and (c) altered influenza virus tropism in the respiratory tracts of co-infected animals. Overall, our study revealed that co-infection with SARS-CoV-2 and influenza virus is associated with altered disease severity and tissue tropism, as well as haematological changes, compared to infection with either virus alone.

KEYWORDS

co-infection, haematological change, influenza A virus, SARS-CoV-2, viral tropism

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1 | INTRODUCTION

Coronavirus disease 2019 (COVID-19) was first detected in late November 2019 and has since been declared a pandemic. Overall, the reported case fatality rate (CFR) of COVID-19 is 1.38%. However, this rate is typically substantially higher (6.4%) in those ≥ 60 years than in those < 60 years (0.32%; Verity et al., 2020). Moreover, these CFRs are considerably higher than those seen in the last influenza pandemic in 2009 (0.048%; Nishiura, 2010), indicating that COVID-19 is a more serious disease. The global significance of COVID-19 outbreaks and the frequent overlap thereof with the influenza season raises concerns about the possibility of co-infection with the causative agent of COVID-19-severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and other respiratory pathogens, particularly influenza viruses (Pinky & Dobrovolsky, 2020). A prior meta-analysis reveals that 3% of hospitalized COVID-19 patients had been co-infected with other respiratory viruses, of which influenza and respiratory syncytial viruses were most common (Cuadrado-Payán et al., 2020; Lansbury et al., 2020). Co-infections are also associated with poorer outcomes, and the related risk of death is 5.9 times that of patients with COVID-19 or influenza alone (Stowe et al., 2020). Co-infection with SARS-CoV and a human reovirus in guinea pigs has resulted in increased diffuse alveolar damage and rapid mortality (Liang et al., 2005). To study the disease caused by co-infection with influenza and SARS-CoV-2 viruses, we used a Syrian hamster model of SARS-CoV-2 and influenza virus infection (Imai et al., 2020; Iwatsuki-Horimoto et al., 2018). We experimentally co-infected Syrian hamsters with SARS-CoV-2 and the pandemic influenza A virus A/California/04/2009 (H1N1) and studied the pathological and haematological outcomes of co-infection, compared to those in animals infected with either virus alone.

2 | MATERIALS AND METHODS

2.1 | Viruses

SARS-CoV-2, BetaCoV/South Korea/KUMC01/2020 (Accession number EPI_ISL_413017, S clade; Park et al., 2020) and the pandemic influenza A virus (Koo et al., 2018)—A/California/04/2009 (H1N1), which was produced using reverse genetics (CA04)—were used in this study. SARS-CoV-2 was purchased from the National Biobank of Korea, passaged three times in VERO-E6 cells (ATCC CRL-1586, American Type Cell Collection), and confirmed by full genome sequencing; then, this stock of virus was used for infection. It was titrated in VERO-E6 cells and cultured in Dulbecco's Modified Eagle's Medium (Thermo Fisher Scientific) supplemented with 2% fetal bovine serum (Thermo Fisher Scientific), penicillin (100 U/ml) and streptomycin (100 $\mu\text{g/ml}$; Thermo Fisher Scientific).

2.2 | Hamster experiments

All animal experiments were approved by the Institutional Animal Care and Use Committee (IACUC) of the Korea Zoonosis Research Institute

at Chonbuk National University (approval number CNUB2020-027). Experiments were conducted in the Biosafety Level 3 facility at the institution, according to standard operating protocols approved by the local Institutional Biosafety Committee. Male Syrian hamsters (7–8 weeks old) were purchased from Central Laboratory Animal Inc. All animals were acclimatized at the BSL-3 facility for at least 5 days prior to the experiments and were randomly allocated into five different experimental groups. Access to food and water was provided ad libitum, and environmental enrichment was provided. Rooms were maintained within set parameters: 20 to 24°C, 45% to 65% humidity and a 12/12 light cycle. Hamsters were anaesthetized with xylazine (10 mg/kg) via intraperitoneal injection prior to intranasal inoculation with 100 μl of 1×10^5 50% tissue culture infective dose (TCID₅₀) of virus and monitored, at least daily, for clinical signs of disease, including survival and weight change. No animals lost more than 30% of their original weight and reached the humane endpoint as defined in the methods during the study. For the co-infection, 1×10^5 TCID₅₀/100 μl of SARS-CoV-2 and CA04 viruses were used for intranasal inoculation, and then the second virus inoculum was given 24 h after the first virus inoculation. To confirm the transmissibility, 24-h post inoculation, naive hamsters were co-housed with the inoculated hamsters for direct contact transmission. To assess viral infectivity and pathogenicity, three hamsters were euthanized by intraperitoneal injection with pentobarbital at a concentration of 200 mg/kg at 2, 4 and 7 days post inoculation (DPI; Figure 1a). The lungs, trachea, nasal turbinates and a kidney were collected from each hamster and homogenized in 1-ml phosphate buffered saline (PBS) with antibiotics to measure viral titers. Lung tissues collected for histopathological analysis were fixed with 4% paraformaldehyde.

2.3 | Viral load determination and immunological profiling

Samples were homogenized in 1 ml of TRIzol Reagent (Invitrogen) before 150 μl of chloroform was added prior to centrifugation at 14,000 rpm for 15 min at 4°C. RNA was extracted using the RNeasy Mini Kit (Qiagen) according to the manufacturer's instructions. Next, 5 μl of extracted RNA was subjected to quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR) to quantify the nucleoprotein gene of SARS-CoV-2 and the matrix gene of influenza A virus using the SensiFAST Probe No-ROX One-Step Kit (Bioline). Viral titers ($\log_{10}\text{TCID}_{50}/\text{ml}$) were determined using a standard curve of the threshold cycle values generated using serially diluted virus (Dovas et al., 2010; Supplementary Figure 1). Immunological profiling of the cytokine response in lung tissue samples was performed using qRT-PCR assays with β -actin mRNA as an internal control and a Syrian hamster-specific primer set (Espitia et al., 2010). For qRT-PCR assays, 2 μl of cDNA was used in a final reaction volume of 20 μl with SYBR Green 2X PCR Master Mix (Enzynomics) and 10 pmol of forward and reverse primers. Cycling conditions were 95°C for 10 min, followed by 40 cycles at 94°C for 10 s and 56°C for 20 s, using a LightCycler 96 System (Roche

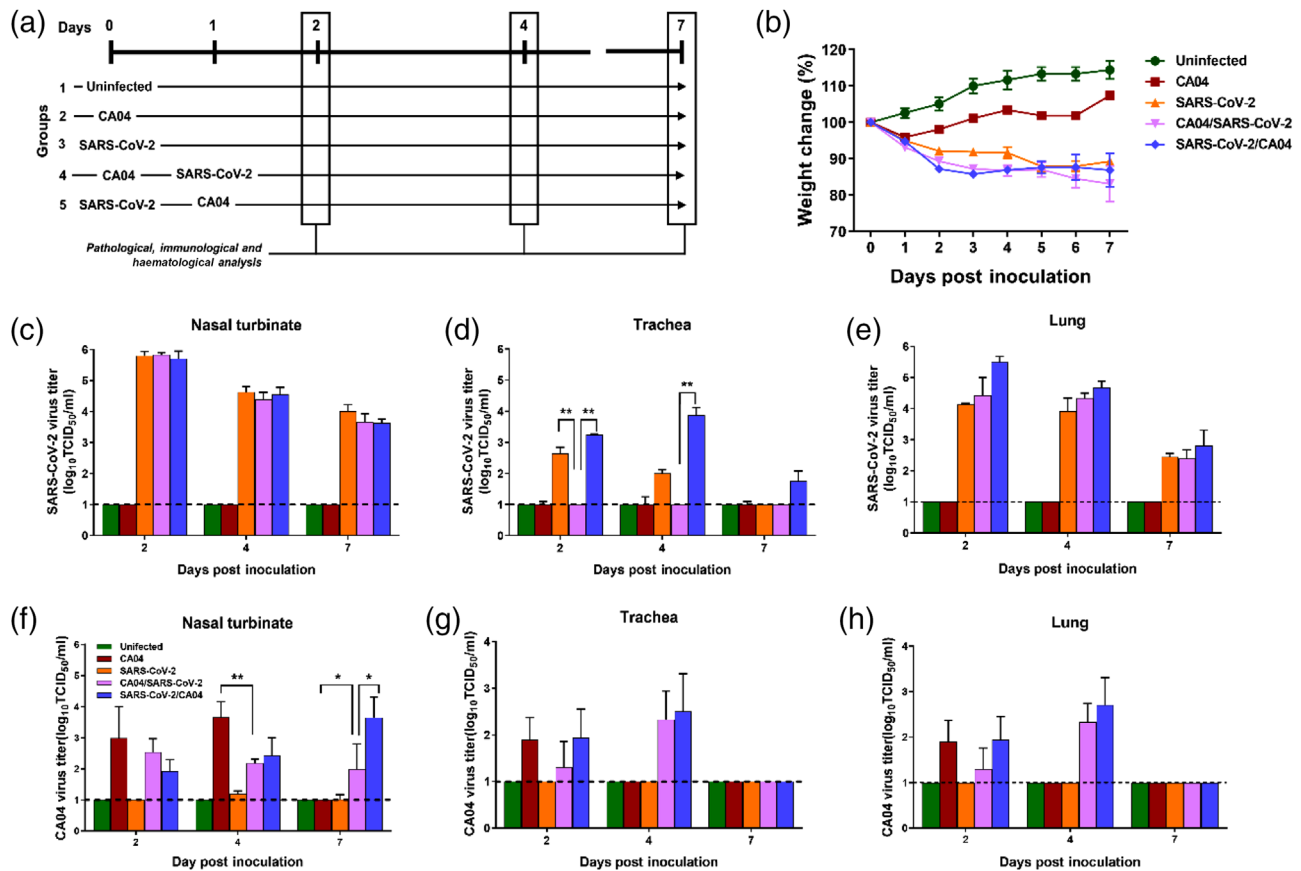


FIGURE 1 Disease severity and tissue tropism in hamsters inoculated or co-inoculated with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and/or CA04. (a) Timeline of experiments showing groups and viruses used to inoculate animals. (b) Mean body weight changes in the experimental groups. Titers of SARS-CoV-2 and CA04 measured in the nasal turbinates (c and f), tracheas (d and g) and lungs (e and h) are shown. The bars and error bars in Panels (c–h) represent the mean \pm standard error of the mean for each group at each time point. The results are shown as the mean \pm s.d. * $p < .05$ and ** $p < .01$

Diagnostics GmbH) according to the manufacturer's instructions. To check primer specificity, melting curves were generated after 40 cycles (Supplementary Table 1 and Supplementary Figure 2). Hamster γ -actin was used as an internal control to normalize and calculate differences in fold change expression, compared to that in uninfected hamsters.

2.4 | Flow cytometry

A single-cell suspension of splenocytes from the spleen and lymphocytes from lung tissue were labelled using the following fluorochrome-conjugated primary antibodies: mouse anti-rat CD8 β (341), rat anti-mouse CD4 (GK1.5) and mouse anti-mouse/rat major histocompatibility complex class II (MHC II I-E k; 14-4-4S; Thermo Fisher Scientific; Hammerbeck & Hooper, 2011). Live cells were stained for 20 min at room temperature and then fixed with 2.5% (w/v) paraformaldehyde. Cells were analysed using a BD Asperse flow cytometer (BD Biosciences) with FACSuite software (BD Biosciences). Data were analysed using FlowJo software (Treestar).

2.5 | Haematological studies

Haematological studies were performed using ethylenediaminetetraacetic acid-treated blood and a Hemavet 950FS analyser (Drew Scientific). Whole blood chemistries were evaluated using heparinized blood and a portable iSTAT blood analyser (Abbott Point of Care) with EC8+ cartridges to measure lactate dehydrogenase.

2.6 | Histopathology and immunohistochemistry

Tissues were fixed in 10% paraformaldehyde and processed for paraffin embedding. Four consecutive sections of 4 μ m each were stained using haematoxylin and eosin (H&E) or monoclonal antibodies specific to the SARS-CoV-2 N protein (NB100-56576, Novus Biologicals) and influenza A virus N protein (NB100-56570, Novus Biologicals), respectively. Images were captured using a Leica DFC 5400 digital camera and processed using Leica Application Suite v.4.13 (Leica Microsystems). Scoring of H&E-stained specimens was conducted according to the following criteria (Yuan et al., 2020): haemorrhages: 1, mild focal haemorrhage; 2, focal haemorrhage with areas distributed throughout the

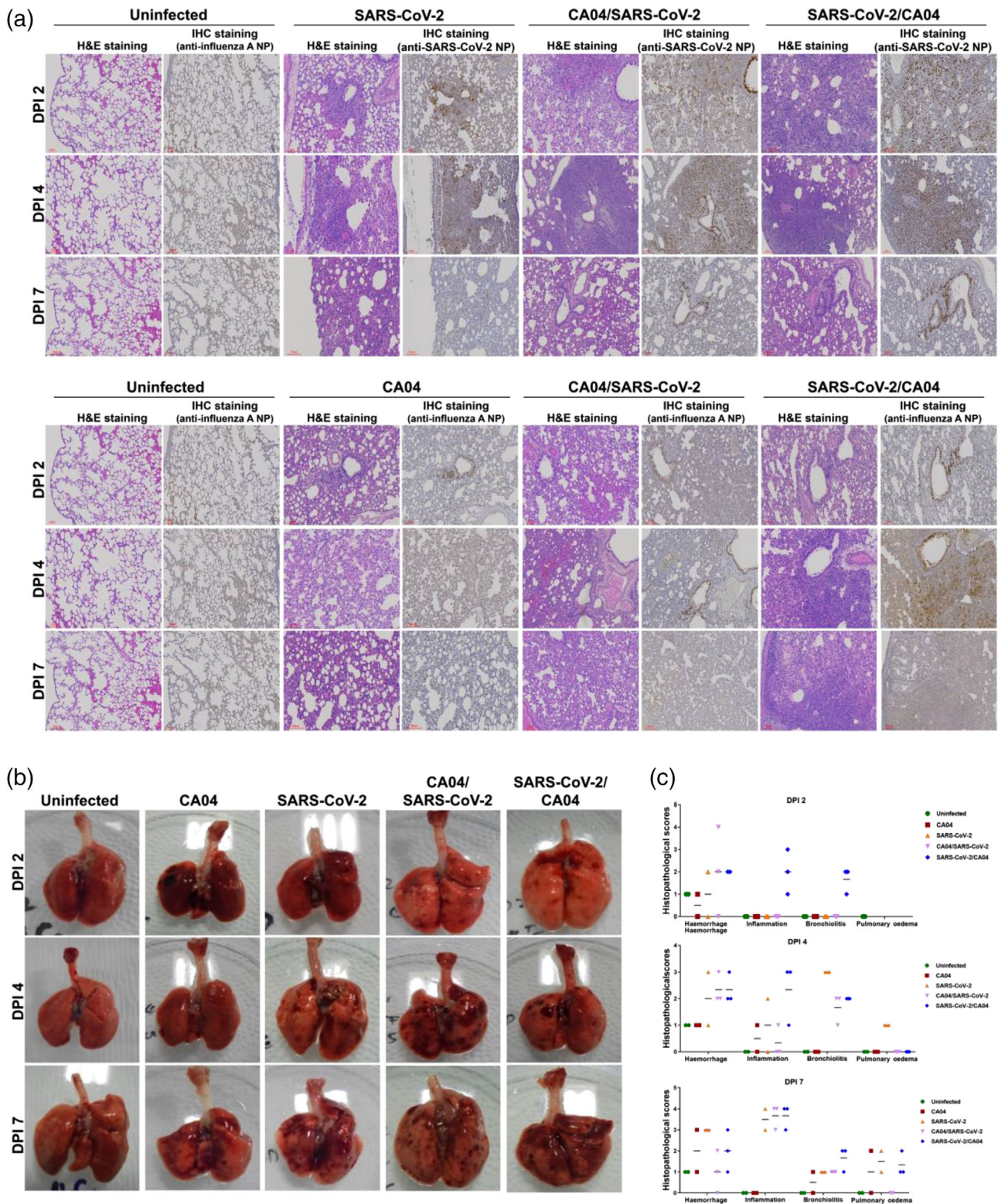


FIGURE 2 Lung pathological changes showing early differences in the disease course of hamsters inoculated or co-inoculated with SARS-CoV-2 and/or CA04. (a) Representative H&E staining and IHC staining with specific antibodies against the SARS-CoV-2 N and influenza A virus N proteins in lung sections obtained from animals at 2, 4 and 7 DPI (scale bar = 100 μ m). (b) Representative gross pathological changes in the lungs of inoculated animals. Lungs are representative of the gross pathological changes observed at 2, 4 and 7 DPI. (c) Pathological changes scoring of haemorrhaging, inflammation, bronchiolitis and pulmonary oedema observed in lung sections. DPI, days post inoculation; H&E, haematoxylin and eosin; IHC, immunohistochemistry

section; 3, confluent haemorrhage throughout the section. Bronchioles: 1, mild peribronchiolar infiltration; 2, peribronchiolar infiltration plus epithelial cell death; 3, score 2 and intra-bronchiolar wall infiltration and epithelium desquamation. Inflammation: 1, alveolar wall thickening and congestion; 2, focal alveolar space infiltration or exudation; 3, diffuse alveolar space infiltration or exudation or haemorrhage. Pulmonary oedema: 1, oedema detected in less than 33% of the lung; 2, oedema detected in 34% to 66% of the lung; and 3, oedema detected in more than 66% of the lung. All tissue samples were assessed by a veterinary anatomic pathologist blinded to study group allocations.

2.7 | Statistical analysis

Differences in viral infectivity, haematological analysis and cytokine/chemokine expression were analysed using the two-tailed Mann–Whitney *U*-test or two-tailed unpaired *t*-test and GraphPad Prism version 5.01 (GraphPad Software Inc.).

3 | RESULTS

3.1 | Compared to inoculation with CA04 alone, but not SARS-CoV-2 alone, co-inoculation resulted in greater disease severity and altered viral tropism

For co-infection of CA04 and SARS-CoV-2, the second virus inoculation sequentially was given 24 h after the first virus inoculation (Zhang et al., 2021). Hamsters were inoculated with CA04 (Groups 2 and 4) or SARS-CoV-2 (Groups 3 and 5) on Day 0, followed by inoculation with CA04 (Group 2), SARS-CoV-2 (Group 3) or mock-inoculation with PBS (Group 1; Figure 1a). Hamsters in all virus-inoculated groups began losing weight on Day 1 post inoculation, but those inoculated only with SARS-CoV-2 lost significantly more weight than CA04-only inoculated animals (Groups 2 and 3; Figure 1b). Weight loss in co-infected animals (Groups 4 and 5) was similar to that observed in the SARS-CoV-2-only inoculated group (Group 3; Figure 1b). Weight loss of the SARS-CoV-2-only ($89.2\% \pm 1.27$), CA04/SARS-CoV-2 ($83.04\% \pm 8.6$) and SARS-CoV-2/CA04 ($86.8\% \pm 7.89$) inoculated group was most severe on Day 7 pi, but the CA04-only inoculated group was recovered to its original weight. No animals lost more than 30% of their original weight and reached the humane endpoint as defined in the methods during the study. These data support that SARS-CoV-2 infection resulted in more severe disease, compared to that caused by CA04, and co-infection did not appear to increase the overall disease severity—measured by weight loss—of SARS-CoV-2. SARS-CoV-2 titers in nasal turbinate and lung tissues were similar across all groups (Figure 1c,e). In tracheal tissue, SARS-CoV-2 titers were only detected in Groups 3 and 5, that is, the groups in which SARS-CoV-2 was inoculated alone or prior to CA04. When CA04 was inoculated before SARS-CoV-2, SARS-CoV-2 titers were not detected in the trachea (Figure 1d). CA04 titers were detected in the nasal turbinate tissues of all co-infected groups (Groups 4 and 5) at 2,

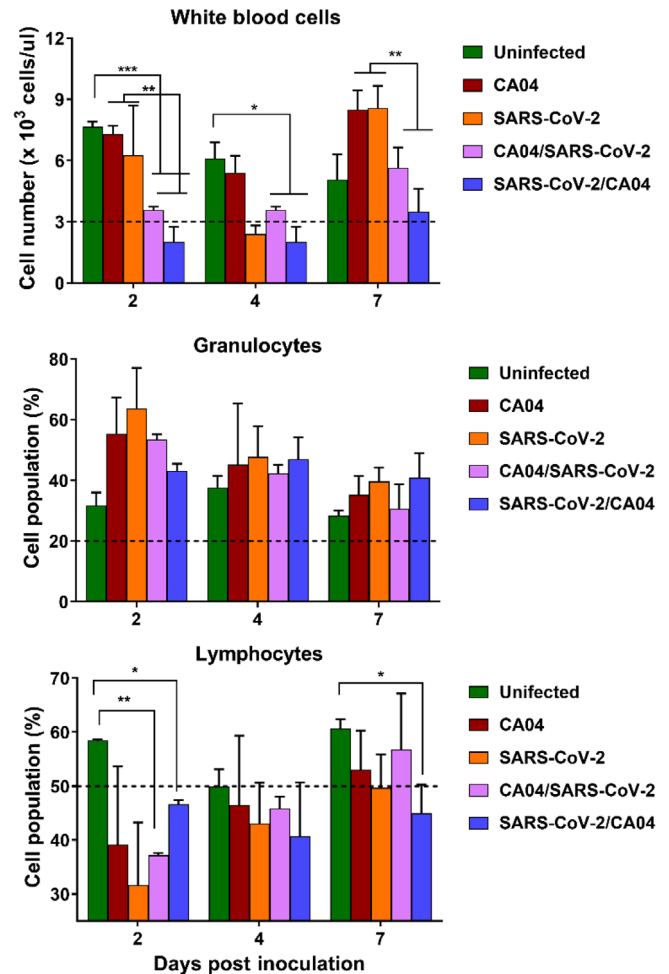


FIGURE 3 Scoring of lung haematology showing early differences in the disease course of hamsters inoculated or co-inoculated with SARS-CoV-2 and/or CA04. Numbers of white blood cells and percentages of the cell population comprising granulocytes and lymphocytes in whole blood samples at 2, 4 and 7 DPI. Bars and error bars represent the mean \pm standard error of the mean for each group at each time point. The results are shown as the mean \pm s.d. * $p < .05$, ** $p < .01$ and *** $p < .001$.

4 and 7 DPI. However, in the CA04-only inoculated group (Group 2), CA04 titers were only detected at 2 and 4 DPI (Figure 1f). A similar pattern was observed in the tracheal (Figure 1g) and lung (Figure 1h) tissues. CA04 titers were detected in the lungs of co-inoculated animals (Groups 4 and 5) at 2 and 4 DPI but only at 2 DPI in animals inoculated with CA04 only (Group 2). This suggests that co-inoculation with SARS-CoV-2 increased the replication—and possibly the tropism—of CA04 in co-inoculated animals, likely resulting in more widespread infection. To confirm transmission by direct contact for both CA04 and SARS-CoV-2, virus titers in lung tissue collected from both donor hamster and contact hamster were assessed. Both viruses were detectable from all contacted hamsters from lung tissues collected at 2 and 4 DPI, but the viruses were completely cleared from lung tissues by 7 DPI (Supplementary Figure 3).

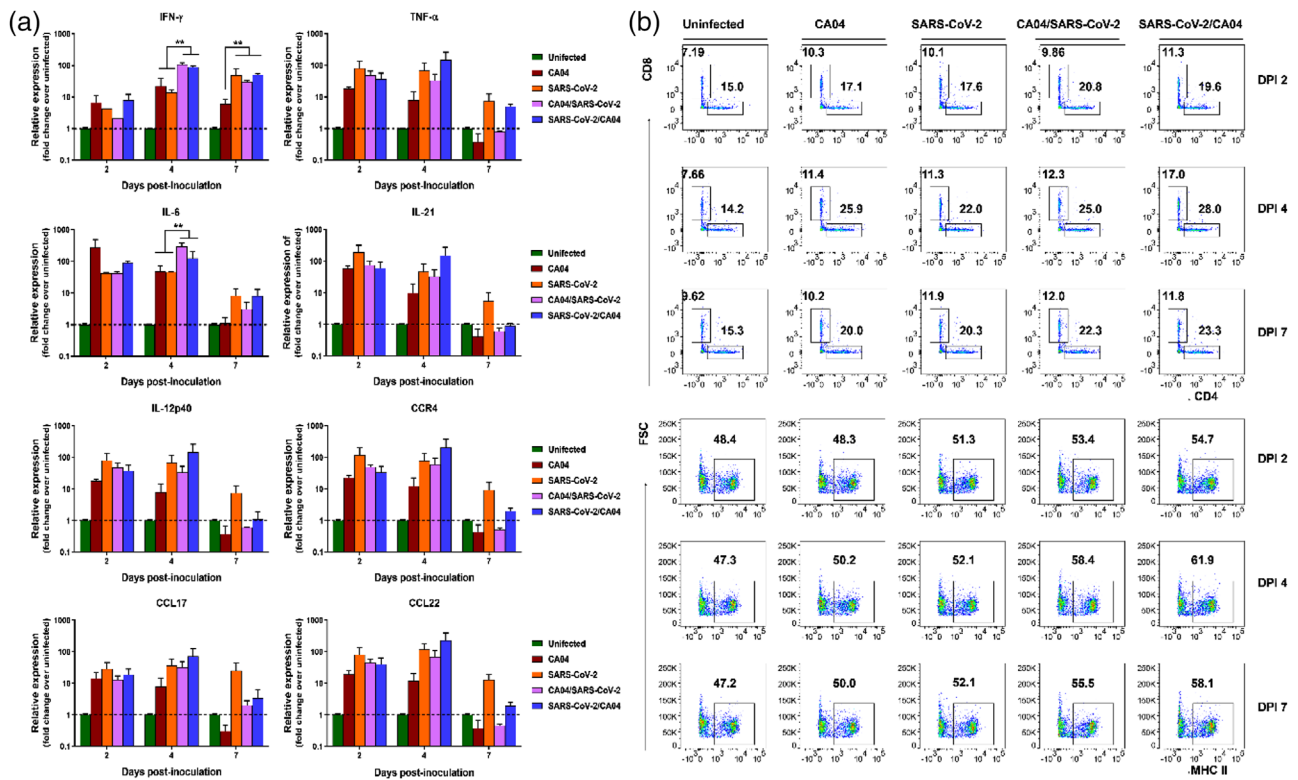


FIGURE 4 Immunological profiling of hamsters inoculated with SARS-CoV-2 and/or CA04. (a) Concomitant upregulation of type 1 and 2 cytokines in the lungs of hamsters inoculated with SARS-CoV-2 and/or CA04. Mean mRNA expression of IFN- γ , TNF- α , IL-6, IL-21, IL-12p40, CCR4, CCL17 and CCL22 at 2, 4 and 7 DPI. Bars and error bars represent the mean \pm standard error of the mean for each group at each time point. The results are shown as the mean \pm s.d. * p < .05 and ** p < .01. (b) T-cell response in the lungs of hamsters inoculated with SARS-CoV-2 and/or CA04. Flow cytometry analysis of the T-cell response in inoculated hamsters. FACS analysis of splenocytes collected at 2, 4 and 7 DPI using specific antibodies targeting CD4+ and CD8+ T cells and B cells. Data were analysed using FlowJo software (Treestar). CCR4, CC chemokine receptor 4; CCL17, C-C motif chemokine ligand 17; IFN- γ , interferon-gamma; TNF- α , tumor necrosis factor-alpha

3.2 | Co-inoculation increased lung pathological changes and was associated with reduced white blood cell and lymphocyte counts and increased pro-inflammatory signalling

Significant lung pathological changes were observed in all inoculated animals at all time points (Figure 2a,b). A greater degree of alveolar interstitial inflammation in the parenchyma, mononuclear cell infiltration and alveolar bronchiolisation, bronchiolitis with surrounding inflammatory cell infiltration (mainly lymphocytes) and luminal exfoliated necrotic cell debris admixed with neutrophils was observed in co-inoculated animals at 2 DPI, but the pathological changes were similar between the inoculated groups at 4 and 7 DPI (Figure 2a,b). These results show that co-infection results in more severe disease at early time points, but this does not translate to greater severity at later time points and is not collinear with the replication kinetics of either virus. Our haematological studies revealed that the numbers of white blood cells (2 and 4 DPI) and lymphocytes (2 and 7 DPI), but not granulocytes, were reduced in co-inoculated animals, compared to animals inoculated with uninfected animals (Figure 3). In particular, white

blood cells were decreased in hamsters co-infected with SARS-CoV-2 and CA04 at 2 and 7 DPI, compared to animals inoculated with SARS-CoV-2 and CA04 (Figure 3). These changes were also associated with altered cytokine expression in the lungs, such as concomitant upregulation of type 1 interferon-gamma (IFN- γ), interleukin 12p40 (IL-12p40) and type 2 cytokines (IL-21), at 2 and 4 DPI. The expression of IFN- γ and IL-6 was significantly higher at 4 DPI in co-inoculated animals and significantly higher at 7 DPI in all SARS-CoV-2 animals, compared to CA04-inoculated animals (Figure 4a). The expression of tumour necrosis factor alpha (TNF- α) was also higher in co-inoculated animals at 2 and 4 DPI (Figure 4a). At 7 DPI, TNF- α expression was higher in animals inoculated with SARS-CoV-2 than in animals inoculated with CA04 only, whose expression was similar to that in uninfected animals (Figure 4b). The expression of IL-21, IL-12p40 and C-C motif chemokine receptor 4 (CCR4) was higher in animals inoculated with SARS-CoV-2 than in those inoculated with CA04 alone (Figure 4a). The expression of C-C motif chemokine ligand 17 (CCL17) and CCL22, which have been implicated in the recruitment of activated Th2 cells and regulatory T cells via CCR4 signalling, was greater in co-inoculated animals at 4 DPI (Figure 4b).

4 | DISCUSSION

The ongoing COVID-19 pandemic and its overlap with influenza seasons could lead to an increased risk of co-infection with SARS-CoV-2 and influenza viruses. However, whether co-infection with both viruses can occur and the possible effects of co-infection on the disease course and severity remain unclear. We used a Syrian hamster model to study infection with SARS-CoV-2 and CA04, as well as in combination. The hamster represents a useful model for such a study, as it is susceptible to both viruses and displays clear clinical signs and pathology (Iwatsuki-Horimoto et al., 2018; Mok et al., 2020). Our data on weight change indicated that there was no synergistic or additive effect of co-infection. The amount of weight lost by co-infected animals was equivalent to that in SARS-CoV-2-only infected animals, whereas less weight loss was observed in the CA04-only infected animals. However, based on lung pathological analysis, the disease severity observed in co-inoculated with CA04 animals concerning lung inflammation. Our model recapitulates the haematological changes observed in patients with COVID-19 (Liao et al., 2020). Coagulopathies are associated with fatal cases, wherein neutrophil-to-lymphocyte ratios and platelet counts serve as exemplary haematological markers of poor prognosis. Here, we found that co-inoculation was associated with worsening haematological characteristics, likely indicating greater disease severity in cases of co-infection. Therefore, haematological support in cases of co-infection may be effective in reducing disease severity.

SARS-CoV-2 co-inoculation also altered the tissue-dependent replication kinetics of CA04, but the impact of CA04 on SARS-CoV-2 replication was less pronounced. Co-inoculation of SARS-CoV-2 (either preceding or following CA04 infection) led to a more rapid detection of CA04 in nasal turbinates and extended the detection period in the trachea and lungs. The factors underlying these observations are not yet clear, but changes in the upper respiratory tract, particularly in the nasal turbinates, might play a role in the transmissibility of these viruses. Our data also showed that co-inoculation could lead to inflammation of the lungs due to lymphopenia and inflammation in the lungs. Interestingly, inoculation with SARS-CoV-2 followed by CA04 resulted in a greater decrease in white blood cells and lymphocytes, compared to that in the other groups. Studies in COVID-19 patients showed that cytokine and chemokine expression was upregulated in the blood of non-severe patients but remained significantly lower than that of severe patients (Huang et al., 2020; Qin et al., 2020). Our study revealed decreased cytokine and chemokine expression at 7 DPI in co-inoculated animals, which correlated with disease severity. Overall, our study revealed that co-infection with SARS-CoV-2 and influenza virus could pose an increased risk of greater disease severity. Early interventions, likely those aimed at correcting haematological changes, may be useful in limiting disease severity.

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CONFLICT OF INTEREST

The authors declare no competing interest.

ETHICS STATEMENT

The experiment was approved by the IACUC of the Korea Zoonosis Research Institute at Chonbuk National University (approval number CNUB2020-027). Experiments were conducted in the Biosafety Level 3 facility at the institution, according to standard operating protocols approved by the local Institutional Biosafety Committee.

DATA AVAILABILITY STATEMENT

Data published in this report are available upon request.

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REFERENCES

- Cuadrado-Payán, E., Montagud-Marrahi, E., Torres-Elorza, M., Bodro, M., Blasco, M., Poch, E., Soriano, A., & Piñeiro, G. J. (2020). SARS-CoV-2 and influenza virus co-infection. *Lancet*, 395(10236), e84. [https://doi.org/10.1016/S0140-6736\(20\)31052-7](https://doi.org/10.1016/S0140-6736(20)31052-7)
- Dovas, C. I., Papanastassopoulou, M., Georgiadis, M. P., Chatzinasiou, E., Maliogka, V. I., & Georgiades, G. K. (2010). Detection and quantification of infectious avian influenza A (H5N1) virus in environmental water by using real-time reverse transcription-PCR. *Applied and Environmental Microbiology*, 76, 2165–2174. <https://doi.org/10.1128/AEM.01929-09>
- Espitia, C. M., Zhao, W., Saldarriaga, O., Osorio, Y., Harrison, L. M., Cappello, M., Travi, B. L., & Melby, P. C. (2010). Duplex real-time reverse transcriptase PCR to determine cytokine mRNA expression in a hamster model of New World cutaneous leishmaniasis. *BMC Immunology*, 11, 31. <https://doi.org/10.1186/1471-2172-11-31>
- Hammerbeck, C. D., & Hooper, J. W. (2011). T cells are not required for pathogenesis in the Syrian hamster model of hantavirus pulmonary syndrome. *Journal of Virology*, 85(19), 9929–9944. <https://doi.org/10.1128/jvi.05356-11>
- Huang, C., Wang, Y., Li, X., Ren, L., Zhao, J., Hu, Y., Zhang, L., Fan, G., Xu, J., Gu, X., Cheng, Z., Yu, T., Xia, J., Wei, Y., Wu, W., Xie, X., Yin, W., Li, H., Liu, M., ... Cao, B. (2020). Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet*, 395, 497–506. [https://doi.org/10.1016/S0140-6736\(20\)30183-5](https://doi.org/10.1016/S0140-6736(20)30183-5)
- Imai, M., Iwatsuki-Horimoto, K., Hatta, M., Loeber, S., Halfmann, P. J., Nakajima, N., Watanabe, T., Ujie, M., Takahashi, K., Ito, M., Yamada, S., Fan, S., Chiba, S., Kuroda, M., Guan, L., Takada, K., Armbrust, T., Balogh, A., Furusawa, Y., ... Kawaoka, Y. (2020). Syrian hamsters as a small animal model for SARS-CoV-2 infection and countermeasure development. *Proceedings of the National Academy of Sciences*, 117, 16587–16595. <https://doi.org/10.1073/pnas.2009799117>

- Iwatsuki-Horimoto, K., Nakajima, N., Ichiko, Y., Sakai-Tagawa, Y., Noda, T., Hasegawa, H., & Kawaoka, Y. (2018). Syrian hamster as an animal model for the study of human influenza virus infection. *Journal of Virology*, 92, e01693–17. <https://doi.org/10.1128/JVI.01693-17>
- Koo, B. -S., Kim, H. K., Song, D., Na, W., Song, M. -S., Kwon, J. J., Wong, S. -S., Noh, J. Y., Ahn, M. -J., Kim, D. -J., Webby, R. J., Yoon, S. -W., & Jeong, D. G. (2018). Virological and pathological characterization of an avian H1N1 influenza A virus. *Archives of Virology*, 163, 1153–1162. <https://doi.org/10.1007/s00705-018-3730-0>
- Lansbury, L., Lim, B., Baskaran, V., & Lim, W. S. (2020). Co-infections in people with COVID-19: A systematic review and meta-analysis. *Journal of Infection*, 81(2), 266–275. <https://doi.org/10.1016/j.jinf.2020.05.046>
- Liang, L., He, C., Lei, M., Li, S., Hao, Y., Zhu, H., & Duan, Q. (2005). Pathology of guinea pigs experimentally infected with a novel reovirus and coronavirus isolated from SARS patients. *DNA and Cell Biology*, 24(8), 485–490. <https://doi.org/10.1089/dna.2005.24.485>
- Liao, D., Zhou, F., Luo, L., Xu, M., Wang, H., Xia, J., Gao, Y., Cai, L., Wang, Z., Yin, P., Wang, Y., Tang, L., Deng, J., Mei, H., & Hu, Y. (2020). Haematological characteristics and risk factors in the classification and prognosis evaluation of COVID-19: A retrospective cohort study. *The Lancet Haematology*, 7(9), e671–e678. [https://doi.org/10.1016/s2352-3026\(20\)30217-9](https://doi.org/10.1016/s2352-3026(20)30217-9)
- Mok, B. W. -Y., Cremin, C. J., Lau, S. -Y., Deng, S., Chen, P., Zhang, A. J., Lee, A. C. -Y., Liu, H., Liu, S., Ng, T. T. -L., Lao, H. -Y., Lee, E. L. -K., Leung, K. S. -S., Wang, P., To, K. K. -W., Chan, J. F. -W., Chan, K. -H., Yuen, K. -Y., Siu, G. K. -H., & Chen, H. (2020). The SARS-CoV-2 spike D614G variant exhibits highly efficient replication and transmission in hamsters. *bioRxiv*. <https://doi.org/10.1101/2020.08.28.271635>
- Nishiura, H. (2010). Case fatality ratio of pandemic influenza. *The Lancet Infectious Diseases*, 10(7), 443–444. [https://doi.org/10.1016/s1473-3099\(10\)70120-1](https://doi.org/10.1016/s1473-3099(10)70120-1)
- Park, S. Y., Kim, Y. -M., Yi, S., Lee, S., Na, B. -J., Kim, C. B., Kim, J. -I., Kim, H. S., Kim, Y. B., Park, Y., Huh, I. S., Kim, H. K., Yoon, H. J., Jang, H., Kim, K., Chang, Y., Kim, I., Lee, H., Gwack, J., ... Jeong, E. K. (2020). Coronavirus disease outbreak in call center, South Korea. *Emerging Infectious Diseases*, 26(8), 1666–1670. <https://doi.org/10.3201/eid2608.201274>
- Pinky, L., & Dobrovolsky, H. M. (2020). SARS-CoV-2 coinfections: Could influenza and the common cold be beneficial? *Journal of Medical Virology*, 92(11), 2623–2630. <https://doi.org/10.1002/jmv.26098>
- Qin, C., Zhou, L., Hu, Z., Zhang, S., Yang, S., Tao, Y., Xie, C., Ma, K., Shang, K., Wang, W., & Tian, D. -S. (2020). Dysregulation of immune response in patients with coronavirus 2019 (COVID-19) in Wuhan, China. *Clinical Infectious Diseases*, 71(15), 762–768. <https://doi.org/10.1093/cid/ciaa248>
- Stowe, J., Tessier, E., Zhao, H., Guy, R., Muller-Pebody, B., Zambon, M., Andrews, N., Ramsay, M., & Lopez Bernal, J. (2021). Interactions between SARS-CoV-2 and influenza, and the impact of coinfection on disease severity: a test-negative design. *International Journal of Epidemiology*, 50(4), 1124–1133. <http://doi.org/10.1093/ije/dyab081>
- Verity, R., Okell, L. C., Dorigatti, I., Winskill, P., Whittaker, C., Imai, N., Cuomo-Dannenburg, G., Thompson, H., Walker, P. G. T., Fu, H., Dighe, A., Griffin, J. T., Baguelin, M., Bhatia, S., Boonyasiri, A., Cori, A., Cucunubá, Z., Fitzjohn, R., Gaythorpe, K., ... Ferguson, N. M. (2020). Estimates of the severity of coronavirus disease 2019: A model-based analysis. *The Lancet Infectious Diseases*, 20(6), 669–677. [https://doi.org/10.1016/s1473-3099\(20\)30243-7](https://doi.org/10.1016/s1473-3099(20)30243-7)
- Yuan, S., Wang, R., Chan, J. F. -W., Zhang, A. J., Cheng, T., Chik, K. K. -H., Ye, Z. -W., Wang, S., Lee, A. C. -Y., Jin, L., Li, H., Jin, D. -Y., Yuen, K. -Y., & Sun, H. (2020). Metalloprotein ranitidine bismuth citrate suppresses SARS-CoV-2 replication and relieves virus-associated pneumonia in Syrian hamsters. *Nature Microbiology*, 5(11), 1439–1448. <https://doi.org/10.1038/s41564-020-00802-x>
- Zhang, A. J., Lee, A. C. -Y., Chan, J. F. -W., Liu, F., Li, C., Chen, Y., Chu, H., Lau, S. -Y., Wang, P., Chan, C. C. -S., Poon, V. K. -M., Yuan, S., To, K. K. -W., Chen, H., & Yuen, K. -Y. (2021). Coinfection by severe acute respiratory syndrome coronavirus 2 and influenza A(H1N1)pdm09 virus enhances the severity of pneumonia in golden Syrian hamsters. *Clinical Infectious Diseases*, 72(12), e978–e992. <https://doi.org/10.1093/cid/ciaa1747>

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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