Replicative Fitness of a SARS-CoV-2 20I/501Y.V1 Variant from Lineage B.1.1.7 in Human Reconstituted Bronchial Epithelium

Franck Touret,[a] Léa Luciani,[a] Cécile Baronti,[a] Maxime Cochin,[a] Jean-Sélim Driouich,[a] Magali Gilles,[a] Laurence Thirion,[a] Antoine Nougairède,[a] Xavier de Lamballerie[a]

[a]Unité des Virus Émergents, Aix-Marseille University, IRD 190, Inserm 1207, Marseille, France

Antoine Nougairède and Xavier de Lamballerie are co-last authors.

ABSTRACT Since its emergence in 2019, circulating populations of the new corona-virus (CoV) continuously acquired genetic diversity. At the end of 2020, a variant named 20I/501Y.V1 (lineage B.1.1.7) emerged and replaced other circulating strains in several regions. This phenomenon has been poorly associated with biological evidence that this variant and the original strain exhibit different phenotypic characteristics. Here, we analyze the replication ability of this new variant in different cellular models using for comparison an ancestral D614G European strain (lineage B1). Results from comparative replication kinetics experiments *in vitro* and in a human reconstituted bronchial epithelium showed no difference. However, when both viruses were put in competition in human reconstituted bronchial epithelium, the 20I/501Y.V1 variant outcompeted the ancestral strain. All together, these findings demonstrate that this new variant replicates more efficiently and may contribute to a better understanding of the progressive replacement of circulating strains by the severe acute respiratory CoV-2 (SARS-CoV-2) 20I/501Y.V1 variant.

IMPORTANCE The emergence of several SARS-CoV-2 variants raised numerous questions concerning the future course of the pandemic. We are currently observing a replacement of the circulating viruses by the variant from the United Kingdom known as 20I/501Y.V1, from the B.1.1.7 lineage, but there is little biological evidence that this new variant exhibits a different phenotype. In the present study, we used different cellular models to assess the replication ability of the 20I/501Y.V1 variant. Our results showed that this variant replicates more efficiently in human reconstituted bronchial epithelium, which may explain why it spreads so rapidly in human populations.

KEYWORDS 20I/501Y.V1, B.1.1.7, SARS-CoV-2, *ex vivo*, *in vitro*, replicative fitness, variant

Novel severe acute respiratory syndrome coronavirus 2 (SARS-Cov-2) emerged in China by the end of 2019 and rapidly spread worldwide. In a few months, the D614G spike mutation was rapidly fixed in almost all circulating SARS-CoV-2 populations, without evidence of higher CoV disease 2019 (COVID-19) mortality or clinical severity (1). It is still being debated whether it is due to a random founder effect (1) or, more probably, whether the mutation enhances viral loads in the upper respiratory tract, increasing the infectivity and stability of virions (2–4).

In September 2020, a variant named 20I/501Y.V1 from lineage B.1.1.7 (initially named VOC 2 2020212/01) emerged in the United Kingdom. It spread rapidly and is becoming dominant in Western Europe (5) and the United States (6). There is consistent epidemiological evidence that this so-called "UK variant" is more efficiently transmitted (7) than the preexisting European strains, in particular in young patients. Moreover, this variant has also been associated in some studies with an increased risk of mortality (8–10), without any differences in symptomatology (11).
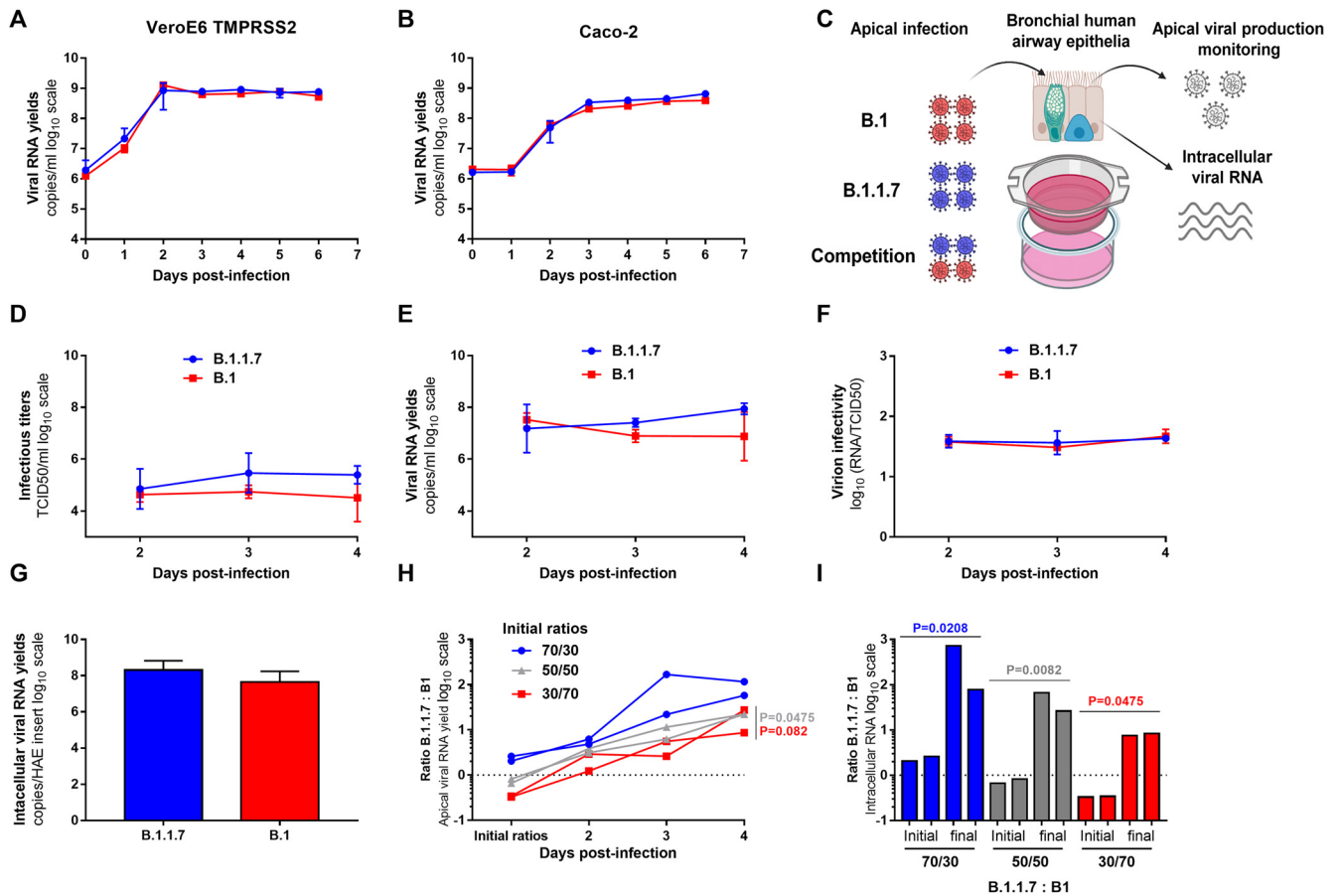
FIG 1 *In vitro* and *ex vivo* replication ability of a 20I/501Y.V1 (B.1.1.7) variant in comparison with a lineage B.1 D614G strain. (A and B) Replication kinetics in VeroE6 TMPRSS2 (A) and Caco-2 (B) cells. Viral replication was assessed using an RT-qPCR assay. (C) Graphical representation of experiments with reconstituted human airway epithelium (HAE) of bronchial origin. (D and E) Kinetics of virus excretion at the apical side of the epithelium measured using a 50% tissue culture infective dose (TCID$_{50}$) assay (D) and an RT-qPCR assay (E). (F) Estimation of virion infectivities (i.e., the ratio of the number of infectious particles to the number of viral RNA particles). (G) Intracellular viral RNA yields measured at 4 dpi using an RT-qPCR assay. (A to G) Data represent means ± standard deviations (SD) from triplicate experiments. No statistical difference was observed between the two viral strains ($P > 0.05$, unpaired Mann-Whitney test). (H) Follow-up of the B.1.1.7/B.1 ratios at the apical side. Each line represents results from an HAE insert. (I) Individual B.1.1.7/B.1 ratios estimated from intracellular viral RNAs at 4 dpi (I). (H and I) P values were determined against the initial ratios using the Kruskal-Wallis test followed by an uncorrected Dunn *post hoc* analysis. The graphical representation was created with BioRender.

Here, we present a comprehensive analysis of the replication ability *in vitro* and *ex vivo* of the 20I/501Y.V1 variant (strain UVE/SARS-CoV-2/2021/FR/7b isolated in February 2021 in Marseille, France; GISAID accession no. EPI_ISL_918165), using for comparison the lineage B.1 BavPat D614G strain that circulated in Europe in February/March of 2020.

The first experiments were performed in two cell lines: VeroE6/TMPRSS2 cells, commonly used for SARS-Cov-2 isolation and propagation (12), and Caco-2 cells, which endogenously express the ACE2 receptor and TMPRSS2 coreceptor at levels similar to those in Calu-3 cells (13). Results of these experiments revealed highly similar replication kinetics, supporting the results of complete genome sequencing of both viral strains with regard to the integrity of the multibasic cleavage site in the spike protein (Fig. 1A and B and see Table S1 in the supplemental material) (14).

We then assessed the replicative fitness of both strains using a previously described model of reconstituted human airway epithelium (HAE) of bronchial origin (15). Following the inoculation of the epithelia through their apical side at a multiplicity of infection (MOI) of 0.1 in order to mimic the natural route of infection, we monitored the excretion of new virions at the apical side between 2 and 4 days postinfection (dpi) and measured the intracellular viral RNA yields at 4 dpi. Infectious titers (Fig. 1D) and viral RNA yields (Fig. 1E) at the apical side at 3 and 4 dpi, as well as intracellular viral

RNA yields at 4 dpi (Fig. 1G), were slightly higher for the B.1.1.7 variant. However, differences were not significant, and estimated relative virion infectivities (i.e., the ratio of the number of infectious particles to the number of viral RNA particles) were similar for the two viruses at all sampling times (Fig. 1F). All together, these results are in line with our findings for common cell lines and with a recent report (16).

Based on these results, we performed competition experiments, which have previously been demonstrated to be effective to detect moderate replicative fitness differences (2, 17). Accordingly, we inoculated epithelia with both viruses simultaneously as described above, sampled the apical side between 2 and 4 dpi, and extracted intracellular viral RNA yields at 4 dpi. Three infection inoculum ratios (B.1.1.7/B1 ratios, 70/30, 50/50, and 30/70) were used. Using two specific reverse transcription-quantitative PCR (RT-qPCR) assays (Fig. S1), we estimated the proportion of each viral genome in the viral population (expressed as the B.1.1.7/B1 ratio in Fig. 1H and I). Regardless of the initial ratio, we observed similar patterns in which B1 was outcompeted by the B.1.1.7 variant; all B.1.1.7/B1 ratio values estimated from apical-side washes were above 1 and over 57, 22, and 8 at 4 dpi for epithelia inoculated at the initial ratios of 70/30, 50/50 and 30/70, respectively (Fig. 1H). Notably, B.1.1.7/B1 ratios measured at 4 dpi were significantly higher than the initial 50/50 and 30/70 inoculum ratios ($P = 0.0475$ and $P = 0.0082$, respectively, with the Kruskal-Wallis test with an uncorrected Dunn *post hoc* analysis). Similar results were observed when estimating the B.1.1.7/B1 ratios from intracellular viral RNAs (Fig. 1I); B.1.1.7/B1 ratios measured at 4 dpi were significantly higher than the initial 50/50 and 30/70 inoculum ratios ($P = 0.0208$, $P = 0.0082$, and $P = 0.0475$ with the 70/30, 50/50, and 30/70 inoculum ratios, respectively, as determined by the Kruskal-Wallis test with an uncorrected Dunn *post hoc* analysis).

Our results demonstrated that the 20I/501Y.V1 (B.1.1.7) variant is more fit than the lineage B.1 BavPat D614G strain in reconstituted bronchial human epithelium. This may be explained by the presence of the N501Y mutation in the receptor binding domain (RBD) of the spike protein, which enhances viral particle binding to the ACE2 receptor (18). This may translate into a fitness advantage, as demonstrated in a recent study with engineered viral strains (19). Similar observations have been made with the D614G mutation, with which the new G614 strains overcame the original D614 strains when put in competition (2). All together, these findings may contribute to a better understanding of the progressive replacement of circulating strains by the SARS-CoV-2 20I/501Y.V1 variant (20).

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.
TEXT S1, DOCX file, 0.02 MB.
FIG S1, DOCX file, 0.2 MB.
TABLE S1, DOCX file, 0.01 MB.
DATA SET S1, DOCX file, 0.04 MB.

REFERENCES

1. Volz E, Hill V, McCrone JT, Price A, Jorgensen D, O'Toole Á, Southgate J, Johnson R, Jackson B, Nascimento FF, Rey SM, Nicholls SM, Colquhoun RM, da Silva Filipe A, Shepherd J, Pascall DJ, Shah R, Jesudason N, Li K, Jarrett R, Pacchiarini N, Bull M, Geidelberg L, Siveroni I, COG-UK Consortium, Goodfellow I, Loman NJ, Pybus OG, Robertson DL, Thomson EC, Rambaut A, Connor TR. 2021. Evaluating the effects of SARS-CoV-2 spike

mutation D614G on transmissibility and pathogenicity. Cell 184:64–75. e11. https://doi.org/10.1016/j.cell.2020.11.020.

2. Plante JA, Liu Y, Liu J, Xia H, Johnson BA, Lokugamage KG, Zhang X, Muruato AE, Zou J, Fontes-Garfias CR, Mirchandani D, Scharton D, Bilello JP, Ku Z, An Z, Kalveram B, Freiberg AN, Menachery VD, Xie X, Plante KS, Weaver SC, Shi P-Y. 2021. Spike mutation D614G alters SARS-CoV-2 fitness. Nature 592:116–116. https://doi.org/10.1038/s41586-020-2895-3.

3. Zhang L, Jackson CB, Mou H, Ojha A, Peng H, Quinlan BD, Rangarajan ES, Pan A, Vanderheiden A, Suthar MS, Li W, Izard T, Rader C, Farzan M, Choe H. 2020. SARS-CoV-2 spike-protein D614G mutation increases virion spike density and infectivity. Nat Commun 11:6013. https://doi.org/10.1038/s41467-020-19808-4.

4. Hou YJ, Chiba S, Halfmann P, Ehre C, Kuroda M, Dinnon KH, Leist SR, Schäfer A, Nakajima N, Takahashi K, Lee RE, Mascenik TM, Graham R, Edwards CE, Tse LV, Okuda K, Markmann AJ, Bartelt L, de Silva A, Margolis DM, Boucher RC, Randell SH, Suzuki T, Gralinski LE, Kawaoka Y, Baric RS. 2020. SARS-CoV-2 D614G variant exhibits efficient replication ex vivo and transmission in vivo. Science 370:1464–1468. https://doi.org/10.1126/science.abe8499.

5. Rambaut A Loman N, Pybus O, Barclay W, Barrett J, Carabelli A, Connor T, Peacock T, Robertson DL, Volz E, on behalf of COVID-19 Genomics Consortium UK. 2020. Preliminary genomic characterisation of an emergent SARS-CoV-2 lineage in the UK defined by a novel set of spike mutations - SARS-CoV-2 coronavirus/nCoV-2019 Genomic Epidemiology. Virological. org. https://virological.org/t/preliminary-genomic-characterisation-of-an-emergent-sars-cov-2-lineage-in-the-uk-defined-by-a-novel-set-of-spike-mutations/563.

6. Galloway SE. 2021. Emergence of SARS-CoV-2 B.1.1.7 lineage — United States, December 29, 2020–January 12. MMWR Morb Mortal Wkly Rep 70:2021. https://www.cdc.gov/mmwr/volumes/70/wr/mm7003e2.htm.

7. Washington NL, Gangavarapu K, Zeller M, Bolze A, Cirulli ET, Barrett KMS, Larsen BB, Anderson C, White S, Cassens T, Jacobs S, Levan G, Nguyen J, Ramirez JM, Rivera-Garcia C, Sandoval E, Wang X, Wong D, Spencer E, Robles-Sikisaka R, Kurzban E, Hughes LD, Deng X, Wang C, Servellita V, Valentine H, Hoff PD, Seaver P, Sathe S, Gietzen K, Sickler B, Antico J, Hoon K, Liu J, Harding A, Bakhtar O, Basler T, Austin B, MacCannell D, Isaksson M, Febbo PG, Becker D, Laurent M, McDonald E, Yeo GW, Knight R, Laurent LC, de Feo E, Worobey M, Chiu CY, et al. 2021. Emergence and rapid transmission of SARS-CoV-2 B.1.1.7 in the United States. Cell 184:2587–2594.e7. https://doi.org/10.1016/j.cell.2021.03.052.

8. Davies NG, Jarvis CI, CMMID COVID-19 Working Group, Edmunds WJ, Jewell NP, Diaz-Ordaz K, Keogh RH. 2021. Increased mortality in community-tested cases of SARS-CoV-2 lineage B.1.1.7. Nature 593:270–274. https://doi.org/10.1038/s41586-021-03426-1.

9. Grint DJ, Wing K, Williamson E, McDonald HI, Bhaskaran K, Evans D, Evans SJ, Walker AJ, Hickman G, Nightingale E, Schultze A, Rentsch CT, Bates C, Cockburn J, Curtis HJ, Morton CE, Bacon S, Davy S, Wong AY, Mehrkar A, Tomlinson L, Douglas IJ, Mathur R, Blomquist P, MacKenna B, Ingelsby P, Croker R, Parry J, Hester F, Harper S, DeVito NJ, Hulme W, Tazare J, Goldacre B, Smeeth L, Eggo RM. 2021. Case fatality risk of the SARS-CoV-2 variant of concern B.1.1.7 in England, 16 November to 5 February. Euro Surveill 26:2100256. https://doi.org/10.2807/1560-7917.ES.2021.26.11.2100256.

10. Challen R, Brooks-Pollock E, Read JM, Dyson L, Tsaneva-Atanasova K, Danon L. 2021. Risk of mortality in patients infected with SARS-CoV-2 variant of concern 202012/1: matched cohort study. BMJ 372:n579. https://doi.org/10.1136/bmj.n579.

11. Graham MS, Sudre CH, May A, Antonelli M, Murray B, Varsavsky T, Kläser K, Canas LS, Molteni E, Modat M, Drew DA, Nguyen LH, Polidori L, Selvachandran S, Hu C, Capdevila J, Hammers A, Chan AT, Wolf J, Spector TD, Steves CJ, Ourselin S, COVID-19 Genomics UK (COG-UK) Consortium. 2021. Changes in symptomatology, reinfection, and transmissibility associated with the SARS-CoV-2 variant B.1.1.7: an ecological study. Lancet Public Health 6:e335–e345. https://doi.org/10.1016/S2468-2667(21)00055-4.

12. Matsuyama S, Nao N, Shirato K, Kawase M, Saito S, Takayama I, Nagata N, Sekizuka T, Katoh H, Kato F, Sakata M, Tahara M, Kutsuna S, Ohmagari N, Kuroda M, Suzuki T, Kageyama T, Takeda M. 2020. Enhanced isolation of SARS-CoV-2 by TMPRSS2-expressing cells. Proc Natl Acad Sci U S A 117:7001–7003. https://doi.org/10.1073/pnas.2002589117.

13. Lee S, Yoon GY, Myoung J, Kim S-J, Ahn D-G. 2020. Robust and persistent SARS-CoV-2 infection in the human intestinal brush border expressing cells. Emerg Microbes Infect 9:2169–2179. https://doi.org/10.1080/22221751.2020.1827985.

14. Lamers MM, Mykytyn AZ, Breugem TI, Wang Y, Wu DC, Riesebosch S, van den Doel PB, Schipper D, Bestebroer T, Wu NC, Haagmans BL. 2021. Human airway cells prevent SARS-CoV-2 multibasic cleavage site cell culture adaptation. Elife 10:e66815. https://doi.org/10.7554/eLife.66815.

15. Pizzorno A, Padey B, Julien T, Trouillet-Assant S, Traversier A, Errazuriz-Cerda E, Fouret J, Dubois J, Gaymard A, Lescure F-X, Dulière V, Brun P, Constant S, Poissy J, Lina B, Yazdanpanah Y, Terrier O, Rosa-Calatrava M. 2020. Characterization and treatment of SARS-CoV-2 in nasal and bronchial human airway epithelia. Cell Rep Med 1:100059. https://doi.org/10.1016/j.xcrm.2020.100059.

16. Brown JC, Goldhill DH, Zhou J, Peacock TP, Frise R, Goonawardane N, Baillon L, Kugathasan R, Pinto AL, McKay PF, Hassard J, Moshe M, Singanayagam A, Burgoyne T, the ATACCC Investigators, PHE Virology Consortium, Barclay WS. 2021. Increased transmission of SARS-CoV-2 lineage B.1.1.7 (VOC 2020212/01) is not accounted for by a replicative advantage in primary airway cells or antibody escape. bioRxiv https://www.biorxiv.org/content/10.1101/2021.02.24.432576v2.

17. de Fabritus L, Nougairède A, Aubry F, Gould EA, de Lamballerie X. 2015. Attenuation of tick-borne encephalitis virus using large-scale random codon re-encoding. PLoS Pathog 11:e1004738. https://doi.org/10.1371/journal.ppat.1004738.

18. Zahradník J, Marciano S, Shemesh M, Zoler E, Chiaravalli J, Meyer B, Rudich Y, Dym O, Elad N, Schreiber G. 2021. SARS-CoV-2 RBD in vitro evolution follows contagious mutation spread, yet generates an able infection inhibitor. bioRxiv https://www.biorxiv.org/content/10.1101/2021.01.06.425392v2.

19. Liu Y, Liu J, Plante KS, Plante JA, Xie X, Zhang X, Ku Z, An Z, Scharton D, Schindewolf C, Menachery VD, Shi P-Y, Weaver S. 2021. The N501Y spike substitution enhances SARS-CoV-2 transmission. bioRxiv https://www.biorxiv.org/content/10.1101/2021.03.08.434499v1.

20. World Health Organization. 2021. Weekly epidemiological update—23 February 2021. World Health Organization, Geneva, Switzerland. https://www.who.int/publications/m/item/weekly-epidemiological-update—23-february-2021.