High replication mutations and fitness of long-term infected SARS-CoV-2 in a man living with HIV

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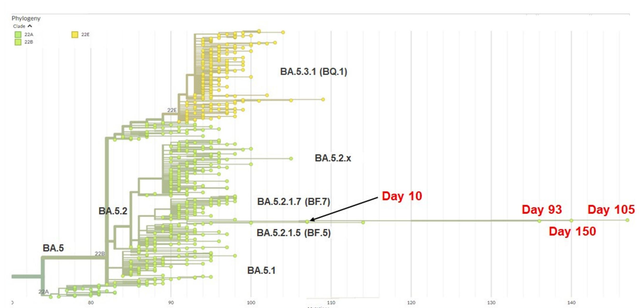
BACKGROUND

In immunocompromised people, including people living with HIV, SARS-CoV-2 infection causes long-term infection and SARS-CoV-2 continues to mutate within the person. In this study, we isolated SARS-CoV-2 from long-term infection in immunocompromised patients and performed virological analysis.

METHODS

SARS-CoV-2 BF.5 (BA.5.2.1.5) was isolated from the nasal swab fluid of a 40-year-old man with HIV (with poor antiretroviral therapy adherence and low CD4 counts) and collected on days 16 and 105 of hospitalization. For the Competitive SARS-CoV-2 Replication Assay (CSRA), viruses from day 16 (SC2 D16) and day 105 (SC2 D105) were co-incubated in VeroE6/TMPRSS2 cells at ratios of 50:50 and 90:10. After 3-4 days, 1/100 volume of supernatant was passaged, and the remaining supernatant was collected to Passage 7. RNA was purified from the collected supernatants, amplified in the spike (S) region via RT-PCR, and sequenced using the MinION Mk1C. For the Viral Replication Assay (VRA), SC2 D16 and SC2 D105 were added to separate cells. After 1 hour, the supernatant was collected, fresh medium was added, and 200 µl of supernatant was collected daily from Day 0 to Day 3. RNA was purified from the collected supernatants, and viral load was measured by RT-qPCR.



Reference Figure. Divergence tree analysis of the whole genome sequence from SARS-CoV-2 BA.5 in Japan. Red indicates the whole-genome sequence of SARS-CoV-2 BF.5 obtained from the patient.

Prolonged SARS-CoV-2 infection in a severely immunocompromised patient living with HIV earned numerous new mutations in the spike region and gained fitness through a high replication rate.

RESULTS

Acquired F157F/L, V213V/G, H245H/N, R346T/K, N440R/K, G446S/D, N450N/D, L452Q/R, E484A/V, F486V, T547T/K, E554D/K and A688A/V mutations in SC2 D16, and acquired T20S, R21S, T33K, S71F, C136F, F157L, V213G, L216F, H245N, R346K, V445A, N450D, G482S, V483del, E484V, T547K, E554K, A570V, P631S and P1143S mutations in SC2 D105 were found in the S region (Table 1). CSRA was analyzed based on the S region 486 of SC2 D16 and SC2 D105, which were V (GTT) 100% and F(TTT) 100%, respectively (Figure 1). The results showed that while SC2 D16 dominated up to P1 in the 60:40 ratio, they tied at P2, SC2 D105 gained the upper hand at P3, and from P5 onward, SC2 D16 accounted for 7% and SC2 D105 for 89%. Similarly, for the 90:10 ratio, SC2 D16 was dominant until P3, but reached parity at P4. SC2 D105 became dominant at P5, and by P7, SC2 D16 was 7% and SC2 D105 was 89% (Figure 2). VRA was significantly higher in SC2 D105 compared to SC2 D16: 72.0-fold higher on Day 1, 150.2-fold higher on Day 2, and 89.1-fold higher on Day 3 (Figure 3).

Table 1. The mutations of the S region in SARS-CoV-2

	20	21	33	71	136	157	213	216	245	346	440	445	446
Wuhan	T	R	T	S	C	F	V	L	H	R	N	V	G
BF.5	T	R	T	S	C	F	G	L	H	R	K	V	G
D16	T	R	T	S	C	F/L	V/G	L	H/N	T/K	R/K	V	S/D
D105	S	S	K	F	F	L	G	F	N	K	K	A	G

	450	452	482	483	484	486	547	554	570	631	688	1143
Wuhan	N	L	G	V	E	F	T	E	A	P	A	P
BF.5	N	R	G	V	A	V	T	E	A	P	A	P
D16	N/D	Q/R	G	V	A/V	V	T/K	D/K	A	P	V/A	P
D105	D	R	S	del	V	F	K	K	V	S	A	S

Green: Receptor Binding Domain (RBD)
Yellow: Receptor Binding Motif (RBM)
Orange: Mutations different from SC2 D16

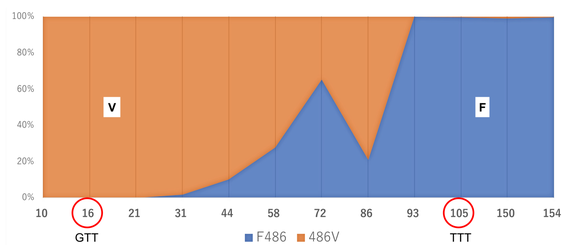


Figure 1. The Evolution of the 486 in the S region. SC2 D16 and SC2 D105 were 100% V (GTT) and 100% F (TTT), respectively.

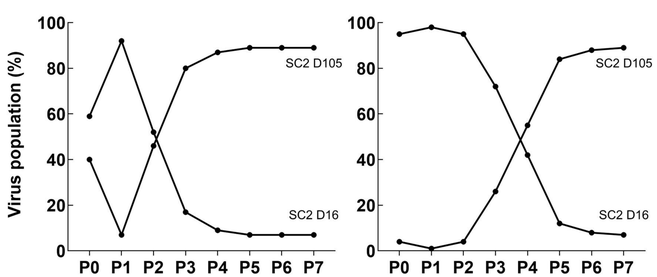


Figure 2. Competitive SARS-CoV-2 Replication Assay (CSRA)

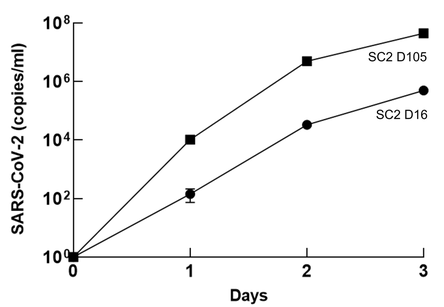


Figure 3. Viral Replication Assay (VRA)

CONCLUSIONS

Both CSRA and VRA results showed that SC2 D105 proliferated more advantageously, suggesting that within patients, SC2 D105 gained fitness and evolved into a virus with mutations distinct from SC2 D16.

ADDITIONAL KEY INFORMATION

Acknowledgements
This work was supported by a Grant for the National Center for Global Health and Medicine (Grant number: 24A1003).

Ethical Approval Statement
This study was approved by the Ethics Committee of the National Center for Global Health and Medicine (approval number: NCGM-S-003595-01).

Reference
Kawashima A, Tsuchiya K, Kuroki K, Nagashima M, Koizumi Y, Nakamoto T, Mizushima D, Aoki T, Sadamasu K, Teruya K, Yoshimura K, Gatanaga H. Prolonged SARS-CoV-2 Infection and Intra-host Viral Evolution in a Severely Immunocompromised Patient with HIV Infection: A Whole-Genome Sequencing Study. Int J Infect Dis, In press.
DOI: 10.1016/j.ijid.2026.108453

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PLAIN LANGUAGE SUMMARY

The long-term persistent infection of SARS-CoV-2 in highly immunocompromised patients acquires fitness and highly replicating mutations within the patient's body.