Virological characteristics of the SARS-CoV-2 JN.1 variant

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Abstract

The SARS-CoV-2 BA.2.86 lineage, first identified in August 2023, is phylogenetically distinct from the currently circulating SARS-CoV-2 Omicron XBB lineages, including EG.5.1 and HK.3. Comparing to XBB and BA.2, BA.2.86 carries more than 30 mutations in the spike (S) protein, indicating a high potential for immune evasion. BA.2.86 has evolved and its descendant, JN.1 (BA.2.86.1.1), emerged in late 2023. JN.1 harbors S:L455S and three mutations in non-S proteins. S:L455S is a hallmark mutation of JN.1: we have recently shown that HK.3 and other "FLip" variants carry S:L455F, which contributes to increased transmissibility and immune escape ability compared to the parental EG.5.1 variant. Here, we investigated the virological properties of JN.1.

Text

The SARS-CoV-2 BA.2.86 lineage, first identified in August 2023, is phylogenetically distinct from the currently circulating SARS-CoV-2 Omicron XBB lineages, including EG.5.1 and HK.3. Comparing to XBB and BA.2, BA.2.86 carries more than 30 mutations in the spike (S) protein, indicating a high potential for immune evasion.[1-4] BA.2.86 has evolved and its descendant, JN.1 (BA.2.86.1.1), emerged in late 2023. JN.1 harbors S:L455S and three mutations in non-S proteins (**Figure 1A**). S:L455S is a hallmark mutation of JN.1: we have recently shown that HK.3 and other "FLip" variants carry S:L455F, which contributes to increased transmissibility and immune escape ability compared to the parental EG.5.1 variant.[5] Here, we investigated the virological properties of JN.1. We estimated the relative effective reproductive number (R_e) of JN.1 using genomic surveillance data from France, the United Kingdom and Spain, where >25 sequences of JN.1 have been reported, using a Bayesian multinomial logistic model (**Figures 1B, 1C, Table S3**).[6] The R_e of JN.1 in these three countries was higher than that of BA.2.86.1 and HK.3, one of the XBB lineages with the highest growth advantage at the end of November 2023 (**Figure 1B**).[5] These results suggest that JN.1 may soon become the dominant lineage worldwide. Indeed, by the end of November 2023, JN.1 has already overtaken HK.3 in France and Spain (**Figure 1C**).

The *in vitro* ACE2 binding assay[7] showed that the dissociation constant (K_D) value of the JN.1 receptor-binding domain (RBD) is significantly higher than that of the BA.2.86 RBD (**Figure 1D**), suggesting that S:L455S decreases the binding affinity to the human ACE2 receptor. In contrast, the pseudovirus assay showed that the infectivity of JN.1 is significantly higher than that of BA.2.86 (**Figure 1E**). This discrepancy (**Figures 1D, 1E**) would be due to the difference between monomeric RBD and trimerized whole S protein (see also **Supplementary Discussion**). We then performed a neutralization assay using rodent sera infected with BA.2.86 or immunized with BA.2.86 S protein. In both cases, the 50% neutralization titer (NT_{50}) against JN.1 was comparable to that against BA.2.86 (**Figures 1F, 1G**), suggesting that S:L455S does not affect the antigenicity of BA.2.86. On the other hand, the NT_{50} of breakthrough infection (BTI) sera with XBB.1.5 and EG.5.1 against JN.1 was significantly lower than that of HK.3 (2.6- to 3.1-fold) and BA.2.86 (3.8-fold) (**Figures 1H, 1I**). Furthermore, JN.1 shows robust resistance to monovalent XBB.1.5 vaccine sera compared to BA.2.86 (**Figure 1J**). Taken together, these results suggest that JN.1 is one of the most immune-evading variants to date. Our results suggest that S:L455S contributes to increased immune evasion, which partly explains the increased R_e of JN.1.

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Declaration of interest

J.I. has consulting fees and honoraria for lectures from Takeda Pharmaceutical Co. Ltd. K.S. has consulting fees from Moderna Japan Co., Ltd. and Takeda Pharmaceutical Co. Ltd. and honoraria for lectures from Gilead Sciences, Inc., Moderna Japan Co., Ltd., and Shionogi & Co., Ltd. The other authors declare no competing interests. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

Figure 1. Virological features of JN.1

(**A**) Frequency of mutations in JN.1 and other lineages of interest. Only mutations with a frequency >0.5 in at least one but not all the representative lineages are shown.

(**B**) Estimated relative R_e of the variants of interest in France, United Kingdom, and Spain. The relative Re of EG.5.1 is set to 1 (horizontal dashed line). Violin, posterior distribution; dot, posterior mean; line, 95% Bayesian confidence interval.

(**C**) Estimated epidemic dynamics of the variants of interest in France, United Kingdom, and Spain from April 1, 2023 to November 16, 2023. Countries are ordered according to the number of detected sequences of JN.1 from high to low. Line, posterior mean, ribbon, 95% Bayesian confidence interval.

(**D**) Yeast surface display affinity between the RBD of the BA.2.86 SARS-CoV-2 variant or BA.2.86 that contained the L455S mutation and mACE2 was measured by yeast surface display. The dissociation constant (K_D) value indicates the binding affinity of the RBD of the SARS-CoV-2 S protein to soluble ACE2 when expressed on yeast. Statistically significant differences versus BA.2.86 is determined by two-sided Student's *t* tests.

(**E**) Lentivirus-based pseudovirus assay. HOS-ACE2/TMPRSS2 cells were infected with pseudoviruses bearing each S protein of B.1.1 or BA.2 sublineages. The amount of input virus was normalized to the amount of HIV-1 p24 capsid protein. The percentage infectivity of B.1.1, BA.2 and JN.1 are compared to that of BA.2.86. The horizontal dash line indicates the mean value of the percentage infectivity of BA.2.86. Assays were performed in quadruplicate, and a representative result of four independent assays is shown. The presented data are expressed as the average ± SD. Each dot indicates the result of an individual replicate. Statistically significant differences versus BA.2.86 is determined by two-sided Student's *t* tests.

(**F**-**J**) Neutralization assay. Assays were performed with pseudoviruses harboring the S proteins of B.1.1, BA.2, BA.2.86, JN.1 and HK.3. The following sera were used: sera from six hamsters infected with BA.2.86 (**F**); sera from ten mice immunized with SARS-CoV-2 BA.2.86 S (**G**); convalescent sera from fully vaccinated individuals who had been infected with XBB.1.5 (eight 3-dose vaccinated donors, six 4-dose vaccinated donors, four 5-dose vaccinated donors and one 6-dose vaccinated donor. 19 donors in total) (**H**); and EG.5.1 (one 2-dose vaccinated donor, four 3-dose vaccinated donors, five 4-dose vaccinated donors, four 5-dose vaccinated donors and four 6-dose vaccinated donors. 18 donors in total) (**I**). Assays were also performed with pseudoviruses harboring the S proteins of BA.2.86 and JN.1. The following two sera were used: vaccinated sera from fully vaccinated individuals who had not been infected (8 donors) and vaccinated sera from fully vaccinated individuals who had been infected with XBB subvariants (after June, 2023) (10 donors). Sera were collected before vaccination ('Pre') and 3-4 weeks after XBB.1.5 monovalent vaccination ('Post') (**J**). Assays for each serum sample were performed in triplicate to determine the 50% neutralization titer (NT_{50}).

Each dot represents one NT_{50} value, and the geometric mean and 95% confidence interval are shown. The number in parenthesis indicates the geometric mean of NT_{50} values. The horizontal dash line indicates the detection limit (40-fold) and the number of samples with neutralization titer under the limit are shown below the dash line. In **F**-**J**, statistically significant differences versus JN.1 were determined by two-sided Wilcoxon signed-rank tests, and p values are indicated in parentheses. The fold changes of NT_{50} from that of JN.1 are indicated with “X”. In **F** and **G**, *, $p<0.05$; **, $p<0.01$ versus JN.1.

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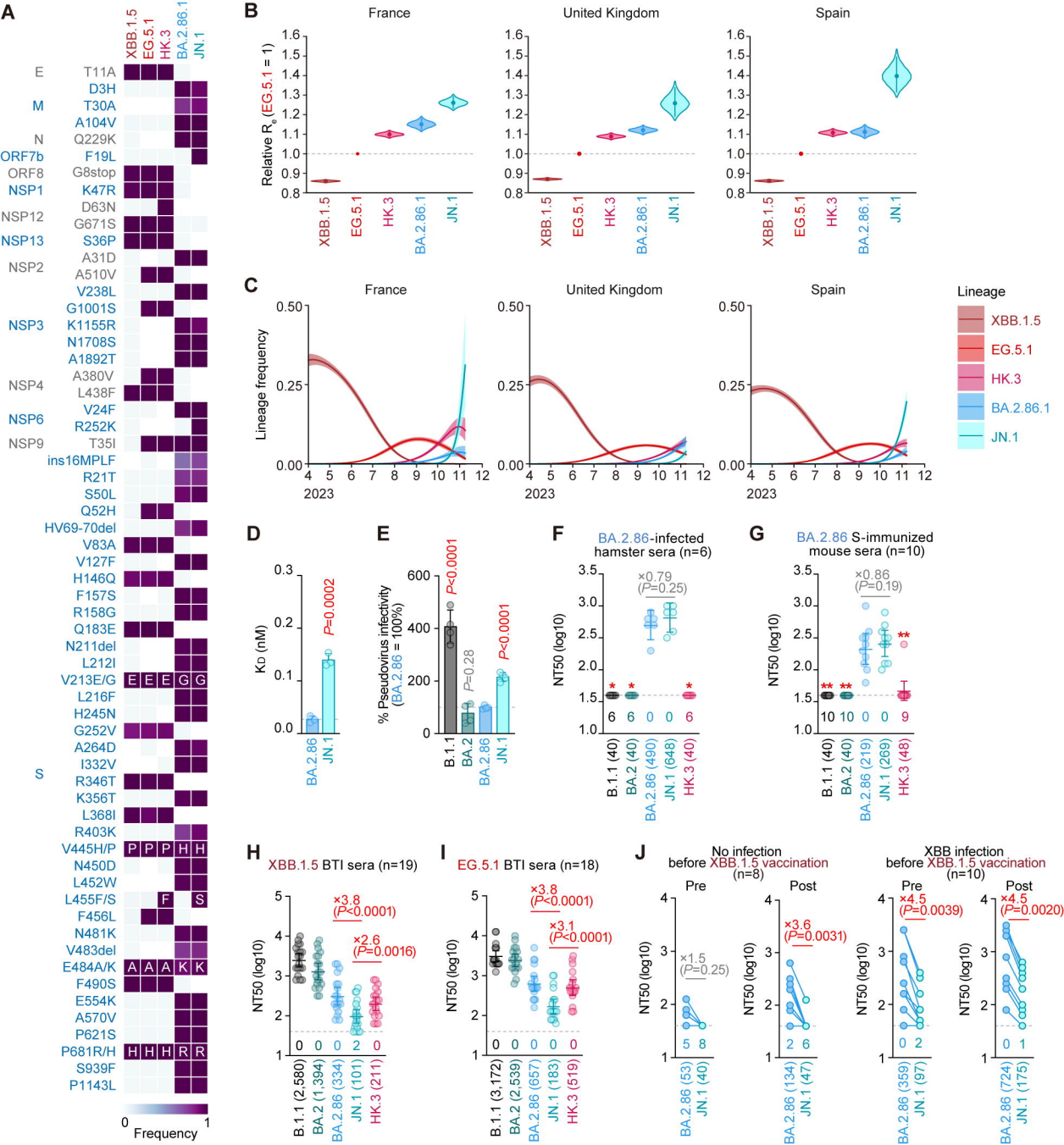
A
XBB.1.5
EG.5.1
HK.3
BA.2.86.1
JN.1
E
T11A
D3H
M
T30A
A104V
N
Q229K
ORF7b
F19L
ORF8
G8stop
NSP1
K47R
NSP12
D63N
G671S
NSP13
S36P
NSP2
A31D
A510V
V238L
G1001S
NSP3
K1155R
N1708S
A1892T
NSP4
A380V
L438F
NSP6
V24F
R252K
NSP9
T35I
ins16MPLF
R21T
S50L
Q52H
HV69-70del
V83A
V127F
H146Q
F157S
R158G
Q183E
N211del
L212I
V213E/G
L216F
H245N
G252V
A264D
I332V
S
R346T
K356T
L368I
R403K
V445H/P
N450D
L452W
L455F/S
F456L
N481K
V483del
E484A/K
F490S
E554K
A570V
P621S
P681R/H
S939F
P1143L
0
Frequency
1
B
France
United Kingdom
Spain
Relative Re (EG.5.1 = 1)
C
Lineage frequency
2023
Lineage
XBB.1.5
EG.5.1
HK.3
BA.2.86.1
JN.1
D
KD (nM)
P=0.0002
BA.2.86
JN.1
E
% Pseudovirus infectivity (BA.2.86 = 100%)
P<0.0001
P=0.28
B.1.1
BA.2
F
BA.2.86-infected hamster sera (n=6)
NT50 (log10)
×0.79 (P=0.25)
B.1.1 (40)
BA.2 (40)
BA.2.86 (490)
JN.1 (648)
HK.3 (40)
G
BA.2.86 S-immunized mouse sera (n=10)
×0.86 (P=0.19)
BA.2.86 (219)
JN.1 (269)
HK.3 (48)
H
XBB.1.5 BTI sera (n=19)
×3.8 (P<0.0001)
×2.6 (P=0.0016)
B.1.1 (2,580)
BA.2 (1,394)
BA.2.86 (334)
JN.1 (101)
HK.3 (211)
I
EG.5.1 BTI sera (n=18)
×3.8 (P<0.0001)
×3.1 (P<0.0001)
B.1.1 (3,172)
BA.2 (2,539)
BA.2.86 (657)
JN.1 (183)
HK.3 (519)
J
No infection before XBB.1.5 vaccination (n=8)
XBB infection before XBB.1.5 vaccination (n=10)
Pre
Post
×1.5 (P=0.25)
×3.6 (P=0.0031)
×4.5 (P=0.0039)
×4.5 (P=0.0020)
BA.2.86 (53)
JN.1 (40)
BA.2.86 (134)
JN.1 (47)
BA.2.86 (359)
JN.1 (97)
BA.2.86 (724)
JN.1 (175)