A novel intranasal administration adenoviral vector-based platform for rapid COVID-19 vaccine development

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ABSTRACT

The coronavirus SARS-CoV-2 has a severe impact on global public health, and the emerging variants threaten the efficacy of the circulating vaccines. Here, we report that a single vaccination with a non-replicated Chimpanzee adenovirus-based vaccine against the SARS-CoV-2 B.1.617.2 variant (JS1-delta) elicits potent humoral, cellular and mucosal immunity in mice. Additionally, a single intranasal administration of JS1-delta provides sufficient protection against B.1.617.2 challenge in mice. This study indicates that Chimpanzee adenovirus type 3 (ChAd3) derived vector represents a promising platform for antiviral vaccine development against respiratory infections, and that JS1-delta is worth further investigation in human clinical trials.

INTRODUCTION

The global coronavirus disease 2019 (COVID-19) pandemic caused by SARS-CoV-2 has severe effects on public health. As of 16 February 2022, there have been over 412 million laboratory-confirmed COVID-19 cases and more than 5.8 million conformed deaths.[1] Although the currently used vaccines have been demonstrated to reduce severe disease and death effectively, the constantly emerging SARS-CoV-2 variants, such as B.1.617.2 (Delta) and B.1.1.529 (Omicron) which have spread quickly and widely around the world, pose serious threats to existing immunologic barrier by compromising the neutralization capability of antibodies and causing breakthrough infection.[2-4] Therefore, rapid response systems for developing vaccines against the pandemic variants should be urgently needed.

Here, we reported the study of a non-replicated Chimpanzee adenovirus-based vaccine against the SARS-CoV-2 delta variant, which is one of the variants of concern (VOC). We evaluated the humoral and cellular immune responses elicited by the candidate vaccine in mice. The induction of mucosal immunity and protective efficacy of the candidate vaccine against B.1.617.2 challenge in mice have also been examined.

RESULTS

Construction and identification of JS1-delta

To develop a prophylactic vaccine against the SARS-CoV-2 delta variant, we constructed a recombinant replication-incompetent adenovirus carrying optimized S-coding sequence, named JS1-delta. Chimpanzee adenovirus type 3 (ChAd3, GenBank ID: CS138463) was chosen to delete E1 region (nt 589-3544) and E3 region (nt 28310-32653). Meanwhile, ChAd3 E4 region (nt 34698-37314) was replaced by the E4 region of human adenovirus type 5 (hAd5, GenBank ID: AY339865, nt 32909-35522). Two endonucleases, I-*Ceu* □ and PI-*Sce* □, were inserted into the original ChAd3 E1 region. This recombinant ChAd3 vector was named JS1 (Fig. 1a). The S-coding sequence from B.1.617.2 was optimized by altering the codon usages to increase its antigen expression in mammalian cells.[5,6] At the same time, the furin cleavage site mutations (R682S, R685G) and proline substitutions (K986P, V987P) were introduced in the sequence to stabilize the S protein and prevent syncytium formation.[7] Then, with the addition of CMV promoter and BGH poly(A) signal, the optimized S-coding sequence was inserted between I-*Ceu* □ and PI-*Sce* □ in JS1 (Fig. 1b). As shown in Fig. 1c, JS1-delta was successfully rescued and multiplied in HEK293 cells, resulting in efficient expression of S protein.

JS1-delta induces potent humoral and cellular immune responses in mice

To assess the immunogenicity of JS1-delta, six-week-old female BALB/c mice (n=5 per group) received a single immunization of 5×10^7 virus particles (VP) (low dose), 5×10^8 VP (middle dose), 5×10^9 VP (high dose) of JS1-delta or 5×10^9 VP of the control vector JS1 via the intramuscular (IM) or intranasal (IN) route at day 0. The titers of delta-S1-specific IgG, neutralizing antibody (NAb) and cellular immune responses were detected in each group. The serum IgG titers were detectable at week 2 and stayed at a high level until week 8 (Fig. 2a). There were higher IgG titers in all three IM groups than in the relative IN groups at week 2, but at week 8 the IN groups showed no significant difference with the IM groups. Similar to the IgG titers, the B.1.617.2 pseudovirus NAb (PNAb) titers were detectable at week 2 and stayed steady until week 8 (Fig. 2b). At week 2, there was no difference between the IN and IM groups, but the PNAb titer in the low-dose IN group was slightly higher than that in the low-dose IM group at week 8, without difference in the middle- and high-dose groups at the same time. Moreover, both the high-dose IN and IM groups elicited potent IFNγ and IL-2 responses in splenic lymphocytes at week 8 (Fig. 2c).

JS1-delta induces effective mucosal immune responses in hACE2 transgenic mice

To further study the capacity of JS1-delta for mucosal immunity induction, 4-week-old female H11-K18-hACE2 mice (hACE2 transgenic C57BL/6J mice, n=6 per group) were immunized intranasally with 5×10^8 VP (low dose) and 5×10^9 VP (high dose) of JS1-delta or the same volume of saline as a control at day 0. The delta-S-specific IgA titers were examined in each group at week 4 post vaccination. As shown in Fig. 2d and 2e, both low- and high-dose groups elicited effective S-specific IgA expressions in the bronchoalveolar lavage fluids (BALFs) and the saliva of mice. Meanwhile, the cross-protections of JS1-delta to SARS-CoV-2 prototype, B.1.1.7 (Alpha), B.1.351 (Beta) and B.1.617.2 variants were also evaluated. As shown in Fig. 2f, the high-dose group showed effective protection to all four SARS-CoV-2 strains at week 4. But compared with those against the delta variant, the NAb titers against other three variants decreased by about 4~6-fold.

JS1-delta protects mice from B.1.617.2 infection

To determine the protective efficacy of JS1-delta through the mucosal vaccination, 4-week-old female H11-K18-hACE2 mice were immunized with 5×10^8 VP (low dose) and 5×10^9 VP (high dose) of JS1-delta by the IN route at day 0. Then, mice were challenged with 1×10^3 PFU of B.1.617.2 virus by nasal drop at day 28 (Fig. 3a). The titers of delta-S-specific serum IgG and NAbs were tested at day -2 and day 26, and all vaccinated mice showed high levels of serum IgG and NAbs, which were not detectable in control animals (Fig. 3b, c). Whereafter, we monitored the weight change and the survival rate in each group. The evaluating indicators kept normal in all groups until day 3 post-infection (3 dpi), but the infected mice without immunization (saline group) lost weight obviously from 4 dpi and all died at 5 dpi, while other groups remained stable and stayed alive (Fig. 3d, e). Subsequently, mice were euthanized at 6 dpi to detect the viral load in lung tissue and for immunofluorescence or pathological examination. As expected, only the saline group exhibited high level of viral RNA copies, displayed lots of SARS-CoV-2 N protein positive cells and showed severe histopathological changes in lung tissue, which were not detectable in vaccinated animals (Fig. 3f, g). Taken together, all these results indicated that both low- and high-dose JS1-delta can confer sufficient protection against B.1.617.2 infection in hACE2 transgenic mice.

DISCUSSION

Multiple reports have found that the SARS-CoV-2 variants can reduce the neutralization capability of monoclonal antibodies (mAbs) and convalescent plasma, and can resist antibody-mediated immunity elicited by vaccines.[4,8-14] As stated by the WHO Technical Advisory Group on COVID-19 Vaccine Composition (TAG-CO-VAC), considering supply and demand of the available vaccines, along with evolution of the virus, repeated booster doses of the original vaccine composition is unlikely to be appropriate or sustainable.[15] Based on the reality that the new variants emerge constantly, it is not enough for vaccines to just reduce hospitalization and mortality, but should be more effective in preventing infection and transmission. In our study, JS1-delta has been demonstrated to protect mice from B.1.617.2 infection sufficiently (Fig. 3). However, its cross-protection against other variants is attenuated (Fig. 2d), which is consistent with another SARS-CoV-2 candidate vaccine JS1-beta (Fig. S1e). These results and reports suggest that a monovalent vaccine used independently may be difficult to handle the complex epidemic situation. Moreover, it is reported that the heterologous prime-boost strategy with COVID-19 vaccines from diverse platforms could be more effective than using a homologous booster.[16,17] Therefore, using heterologous boosters or developing multivalent vaccines containing various SARS-CoV-2 variants compositions may be more efficient and practical to fight against the virus.

Replication deficient recombinant adenoviral vectors possess great immunogenicity and can elicit potent cellular immune responses in preclinical and clinical researches, in which hAd5 is the most comprehensively studied.[18-22] However, hAd5 shows high seroprevalence in global populations due to natural infection, especially in the developing world, and high level of the pre-existing immunity could result in decreased efficacy of the hAd5-based vaccines.[18,19] Meanwhile, considering that HEK293 remains a major producer cell line for adenovirus development and manufacturing, replication-competent adenovirus (RCA) contamination is a significant trouble for large-scale production and clinical application of the hAd5-derived vectors.[23,24] To circumvent these limitations, considerable efforts have been made to develop new adenoviral vectors from lower seroprevalence hAds (such as hAd26, hAd28 and hAd35)[18,25,26] or from non-human primate sources (such as ChAdY25 and ChAd63)[27,28]. ChAd3 is one of the most promising candidates and shows well safety and tolerance in clinical trials targeting hepatitis C virus and Ebola virus.[29-32] In this study, we have demonstrated for the first time, to our knowledge, that a ChAd3-derived SARS-CoV-2 vaccine can induce potent humoral and cellular immune responses and completely protect mice from B.1.617.2 challenge. At the same time, another vaccine candidate JS1-beta targeting B.1.351 shows favorable immunogenicity in mice (Fig. S1), and JS1-omicron targeting B.1.1.529 is also under testing. The longevity of antigen-specific immune responses and the influence of anti-vector immunity induced by the vaccines, along with the cooperation with different boosters, will be further investigated.

Recently, mucosal vaccines against SARS-CoV-2 have raised increasing interests due to their important advantage in preventing virus replication in the upper respiratory tracts, which is essential to interrupt pulmonary infection and person to person transmission.[33-36] Compared with IM injection, the IN vaccination can elicit not only comparable systemic immunity but also additional pathogen-specific mucosal immunity, resulting in a first line of defense against the virus invasion.[34-36] In our study, IN vaccination of JS1-delta induces S-specific IgA expressions efficiently in both BALFs and saliva of the hACE2 transgenic mice (Fig. 2e, f). Using SARS-CoV-2 as a model, our findings both *in vitro* and *in vivo* highlight that the non-replicating ChAd3-derived vector administrated in the IN route represents a powerful platform for rapid vaccine development against respiratory virus infections.

MATERIALS AND METHODS

Construction of JS1-delta

The chemosynthetic JS1 genome was cyclized by the additional pUC57 backbone and was linearized by a double enzyme digestion with I-*Ceu* □ and PI-*Sce* □. Then, the chemosynthetic delta-*S* sequence was inserted into JS1 genome by homologous recombination, and JS1-delta genome was released from the backbone by the digestion with P*ac* □. Finally, HEK293 (ATCC) cells were transfected with linear JS1-delta genome to rescue the recombinant virus.

Animals

The experiments involving animals were approved by and carried out in accordance with the guidelines of the Institutional Experimental Animal Welfare and Ethics Committee. Specific pathogen-free female BALB/c mice were obtained from Charles River Laboratories. Specific pathogen-free female H11-K18-hACE2 mice were obtained from GemPharmatech.

Purification of SARS-CoV-2 delta-S protein

SARS-CoV-2 delta-S protein (1-1141aa) with a C-terminal 6×His tag was expressed in HEK293 cells. In brief, adherent HEK293 cells were transfected with recombinant pCDNA3.1-delta S-His (1-1141) using PEI and incubated in 37℃, 5% CO_2 for 72 h. The supernatant was harvested by centrifugation and purified by Ni-NTA affinity chromatography.

ELISA

The antigen expression in JS1-delta infected HEK293 cells was examined by SARS-CoV2 (2019-nCoV) Spike RBD ELISA KIT (SinoBiological) following the manufacturer’s instruction. To measure the titers of SARS-CoV-2-specific IgG or IgA, 96-well clear polystyrene microplates (Corning) were coated with delta-S1 protein (GenScript) or purified delta-S protein and incubated at 4 °C overnight. After washes for 2-4 times, the plates were blocked at 37 °C for 1 h with 5% skim milk in PBS and washed again with PBST. The plates were then added with 100 μL serially diluted sera or BALFs or saliva and incubated at 37 °C for 1 h. Following washes, HRP-conjugated Affinipure Goat Anti-Mouse IgG (H+L) (Proteintech, 1:5000 dilution) or Goat Anti-Mouse IgA-HRP (SouthernBiotech, 1:5000 dilution) was added to the plates and incubated for 1 h at 37 °C. The assays were developed with TMB substrate (Sigma), stopped by the addition of 2M H_2SO_4 and were measured at 450 nm/630 nm. The endpoint titre was defined as the highest dilution yielding an absorbance >2-fold over the values of negative control.

Neutralization assay

To test SARS-CoV-2 PNAbs, serial dilutions of heat-inactivated sera were mixed with B.1.617.2 pseudoviruse (Beijing Tiantan Pharmacy Biological Research & Development Company), incubated for 1 h at 37 °C and added to ACE2-293T (YEASEN) cells in duplicate in 96-well microplate. Cells were lysed 24 h later and luciferase activity was measured. The neutralization titers were calculated as the serum dilution at which the luciferase activity reduced by 50% compared with the virus-only wells.

To test SARS-CoV-2 NAbs, serial dilutions of heat-inactivated sera were incubated with 70 PFU of the indicated variants (isolated and stored by Wuhan Institute of Virology, CAS) at 37 °C for 1 h. The mixtures were added to pre-plated Vero E6 (ATCC) cell monolayers in 96-well microplate and cultured for 3-4 day. NAb titres were calculated by Reed Muench method to estimate the serum dilution required for a 50% CPE reduction.

ELISpot

SARS-CoV-2-specific cellular immune responses were assessed by the IFNγ and IL-2 ELISpot Kit (Cellular Technology Limited) following the manufacturer's instructions. In brief, 1×10^6 of mice splenic lymphocytes were stimulated with delta-S protein in a pre-coated ELISpot plate for 16 h at 37 °C. After being washed twice, the plates were added with Anti-murin IFNγ Detetion Solution or Anti-murin IL-2 Detetion Solution and incubated at room temperature for 2 h. The plates were then added with Tertiary Solution and Blue Developer Solution. Finally, the spots were counted with ImmunoSpot Analyzer (CTL S6 uNVERSAL).

Quantitative RT-PCR

Viral RNA was extracted using TRI Reagent (Sigma). Reverse transcription was performed using HiScript II Q RT SuperMix for qPCR (Vazyme). Subsequently, 5 µl cDNA was added into a 25 µl qRT-PCR reaction containing ChamQ SYBR qPCR Master Mix (Vazyme). The primers designed to target the nucleocapsid protein of SARS-CoV-2 were (forward) 5’-GGG GAA CTT CTC CTG CTA GAA T-3’ and (reverse) 5’-CAG ACA TTT TGC TCT CAA GCT G-3’. The samples were run in triplicate on an ABI 7900 Real-Time System (Applied Biosystems, Thermo). The following cycling conditions were performed: 1 cycle of 50 °C for 2 min, 1 cycle of 95 °C for 10 min, and 40 cycles of 95 °C for 15 sec and 58 °C for 1 min. Finally, the amount of viral RNA was normalized to the standard curve from a plasmid containing the full-length SARS-CoV-2 *N* gene.

IF and H&E

The lung tissues were immersed in 10% neutral buffered formalin (Sigma) for 24 h. After the formalin fixation, the tissues were placed in 70% ethanol (Merck) and subsequently embedded with paraffin. Tissue sections (5-µm thick) were prepared for H&E staining or immunofluorescence staining using SARS-CoV/SARS-CoV-2 Nucleocapsid Antibody (SinoBiological). Images were collected under a Pannoramic MIDI system (3DHISTECH, Thermo) using Pannoramic scanner software and analyzed by ImageJ (NIH).

Statistical analysis

Data are presented as the mean ± standard error of the mean. Statistics were performed using the nonparametric Mann-Whitney U test or the Student t test. $P<0.05$ was considered statistically significant (* $p<0.05$, ** $p<0.01$).

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AUTHOR CONTRIBUTIONS

M.L. conceived this project. Y.Y. designed the study and wrote the manuscript with input from all authors. W.X., C.H. and B.L. constructed the virus and performed the experiment in BALB/c mice. W.F. performed the protein purification and ELISpot. Q.S. designed the challenge study. F.N., H.H., Y.Li, Q.H. and Y.Liu evaluated the efficacy of JS1-delta in B.1.617.2 infected transgenic hACE2 mice. X.G. and G.Z. coordinated the project.

Competing interests: The authors declare no competing interests.

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FIGURE LEGENDS

Fig.1 Construction and identification of JS1-delta. a, b Schematic of JS1 and JS1-delta genome. CMV: cytomegalovirus promoter. RBD: receptor-binding domain of SARS-CoV-2 spike protein. BGH: bovine growth hormone poly(A) signal. **c** HEK293 cells were infected with JS1-delta and then cultured for 48 hours. The cell supernatant was collected to detect the expression of S protein by ELISA.

Fig.2 The immunogenicity of JS1-delta in mice. a-c 6-week-old BALB/c mice (n=5 per group) were vaccinated with JS1-delta or the control vector (JS1) using the indicated dosage by the IN or IM route at day 0. The titers of S1-specific IgG, B.1.617.2 PNAb and the levels of cellular immune responses at week 8 were detected by ELISA, luciferase assay and ELISpot, respectively. **d-f** 4-week-old H11-K18-hACE2 mice (n=6 per group) were immunized intranasally with 5×108 VP (low dose) and 5×109 VP (high dose) of JS1-delta or the same volume of saline at day 0. At week 4, the S-specific IgA titers in BALFs or saliva were examined by ELISA. The NAb titers against the indicated SARS-COV-2 strains were detected by CPE calculation.

Fig.3 JS1-delta protects mice from B.1.617.2 infection. a Schematic of experimental strategy and grouping. **b, c** 4-week-old H11-K18-hACE2 mice were immunized intranasally with 5×108 VP (low dose, n=10) and 5×109 VP (high dose,

n=10) of JS1-delta or the same volume of saline (n=6) at day 0. Then, the mice were challenged with 1×103 PFU of B.1.617.2 virus in a volume of 50 μL by nasal drop at day 28. Serums were collected at day -2 and day 26, and the titers of S-specific IgG and B.1.617.2 NAb were detected by ELISA and CPE calculation. **d** The weight change was monitored every week after vaccination and every day after infection. **e** The survival rate was monitored every day after infection. **f, g** The mice in saline group died at 5dpi, the others were sacrificed at 6dpi. The viral load in lung tissue was detected by qPCR. SARS-COV-2 N protein expression and histopathological examinations in lungs were examined by immunofluorescence (IF) and hematoxylin-eosin staining (H&E).

Fig.1

a
I-*Ceu* I
PI-*Sce* I
hAd5 E4
delete E1
delete E3
JS1
b
I-*Ceu* I
PI-*Sce* I
S
hAd5 E4
delete E3
I-*Ceu* I
PI-*Sce* I
CMV
RBD
BGH
T19R
G142D
E156G
Δ157-158
L452R
T478K
D614G
P681R
R682S
R685S
D950N
K986P
V987P
Delta-S
c
S expression (pg/ml)
2000
1500
1000
500
0

Fig.2

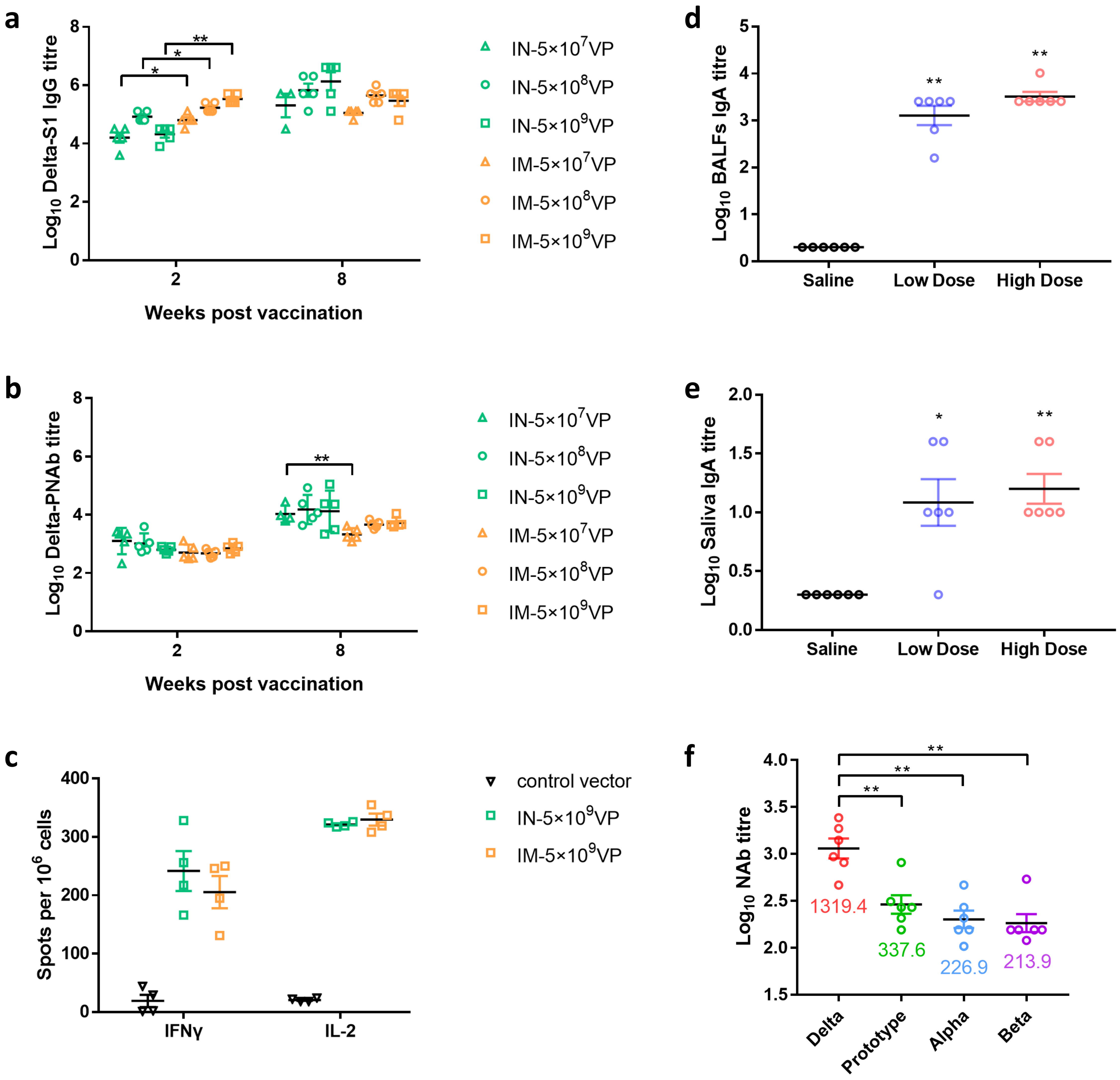
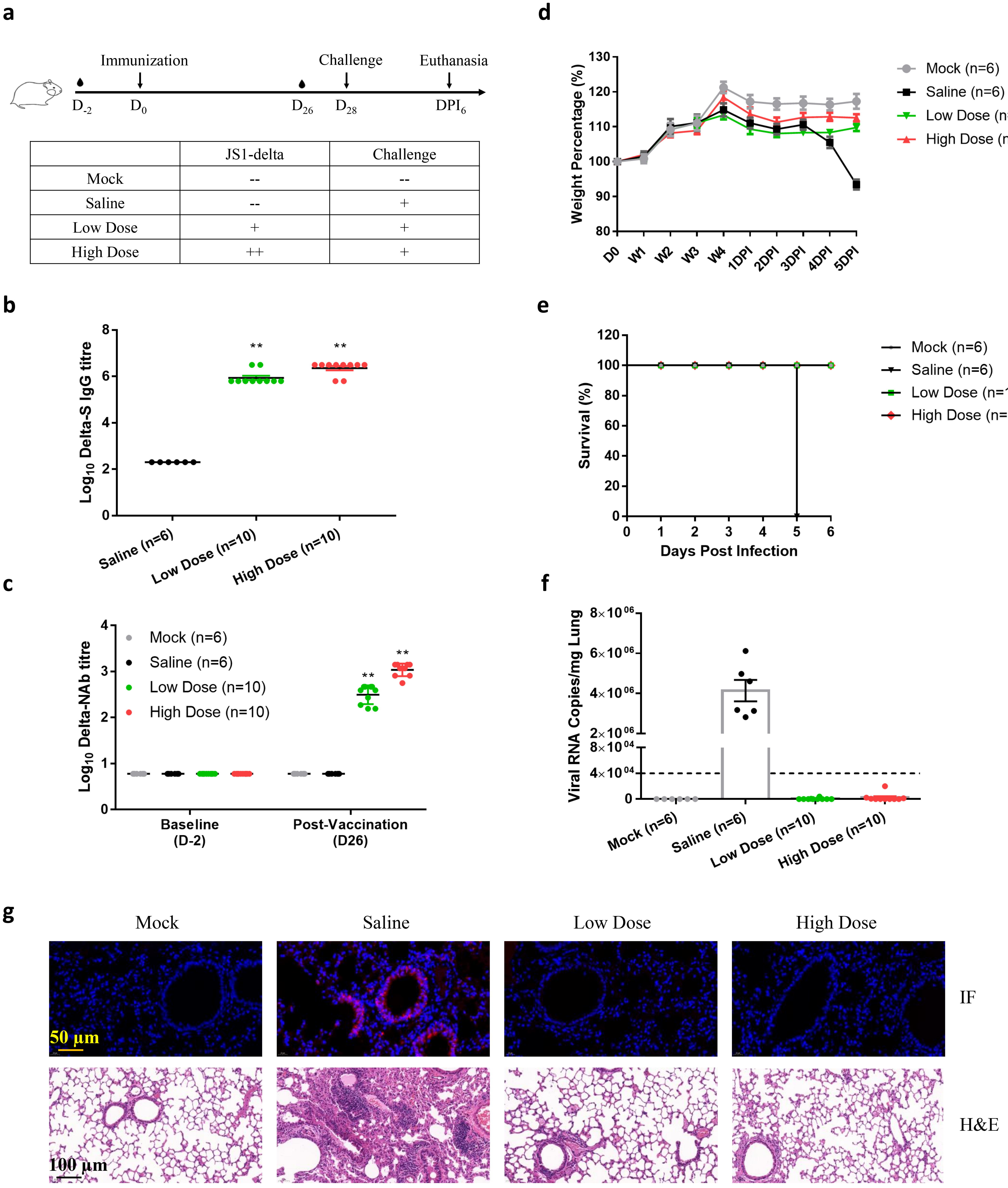

a
Log10 Delta-S1 IgG titre
0
2
4
6
8
*
*
**
2
8
Weeks post vaccination
IN-5×10^7VP
IN-5×10^8VP
IN-5×10^9VP
IM-5×10^7VP
IM-5×10^8VP
IM-5×10^9VP
b
Log10 Delta-PNAb titre
0
2
4
6
8
**
2
8
Weeks post vaccination
IN-5×10^7VP
IN-5×10^8VP
IN-5×10^9VP
IM-5×10^7VP
IM-5×10^8VP
IM-5×10^9VP
c
Spots per 10^6 cells
0
100
200
300
400
IFNγ
IL-2
control vector
IN-5×10^9VP
IM-5×10^9VP
d
Log10 BALFs IgA titre
0
1
2
3
4
5
**
**
Saline
Low Dose
High Dose
e
Log10 Saliva IgA titre
0.0
0.5
1.0
1.5
2.0
*
**
Saline
Low Dose
High Dose
f
Log10 NAb titre
1.5
2.0
2.5
3.0
3.5
4.0
**
**
**
1319.4
337.6
226.9
213.9
Delta
Prototype
Alpha
Beta

Fig.3



	JS1-delta	Challenge
Mock	--	--
Saline	--	+
Low Dose	+	+
High Dose	++	+