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Article

ChAdOx1 nCoV-19 vaccine prevents SARS-CoV-2 pneumonia in rhesus macaques

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged in December 2019[1,2] and is responsible for the COVID-19 pandemic[3]. Vaccines are an essential countermeasure urgently needed to control the pandemic[4]. Here, we show that the adenovirus-vectored vaccine ChAdOx1 nCoV-19, encoding the spike protein of SARS-CoV-2, is immunogenic in mice, eliciting a robust humoral and cell-mediated response. This response was predominantly Th1, as demonstrated by IgG subclass and cytokine expression profiling. Vaccination with ChAdOx1 nCoV-19 (prime-only and prime-boost regimen) induced a balanced Th1/Th2 humoral and cellular immune response in rhesus macaques. We observed a significantly reduced viral load in bronchoalveolar lavage fluid and lower respiratory tract tissue of vaccinated rhesus macaques challenged with SARS-CoV-2 compared with control animals, and no pneumonia was observed in vaccinated animals. However, there was no difference in nasal shedding between vaccinated and control animals. Importantly, no evidence of immune-enhanced disease following viral challenge in vaccinated animals was observed. Safety, immunogenicity and efficacy of ChAdOx1 nCoV-19 against symptomatic PCR-positive COVID-19 disease will now be assessed in randomised controlled human clinical trials.

ChAdOx1 is a replication-deficient simian adenoviral vector derived from isolate Y25. The seroprevalence of antibodies to Y25 in the human population was found to be 0% in the United Kingdom and 9% in the Gambia[5]. We previously demonstrated that a single dose of ChAdOx1 MERS, encoding the spike protein of MERS-CoV, protected non-human primates (NHPs) against MERS-CoV-induced disease[6]. Upon the emergence of SARS-CoV-2, we designed a similar ChAdOx1-vectored vaccine encoding a codon-optimized full-length SARS-CoV-2 spike protein.

Immunogenicity in mice

Two mouse strains (BALB/c, N=5 and outbred CD1, N=8) were vaccinated intramuscularly (IM) with ChAdOx1 nCoV-19 or ChAdOx1 GFP, a control vaccine encoding green fluorescent protein. Humoral and cellular immunity were studied 9-14 days later. Total IgG titers were detected against spike protein in all vaccinated mice (Figure 1a). Profiling of the IgG subclasses showed a predominantly Th1 response post vaccination (Extended Data Figure 1a) and induction of IgM antibodies (Extended

Data Figure 2a). Virus-specific neutralising antibodies were detected in all mice vaccinated with ChAdOx1 nCoV-19, whereas no neutralisation was detected in serum from mice vaccinated with ChAdOx1 GFP (Figure 1b). Splenic T-cell responses measured by IFN-γ ELISpot and intracellular cytokine staining (ICS) were detected against peptides spanning the full length of the spike construct (Figure 1c). Again, a strong Th1-type response was detected post vaccination as supported by high levels of IFN-γ and TNF-α, and low levels of IL-4 and IL-10 (Figure 1d & Extended Data Figure 1b, c).

Immunogenicity in rhesus macaques

We next evaluated the efficacy of ChAdOx1 nCoV-19 in rhesus macaques, a non-human primate model that displays robust infection of upper and lower respiratory tract and virus shedding upon inoculation with SARS-CoV-2[7]. Six animals per group were vaccinated via a prime-only regimen (28 days before challenge) or a prime-boost regimen (56 and 28 days before challenge) intramuscularly with 2.5 x 10^10 ChAdOx1

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nCoV-19 virus particles each. As a control, six animals were vaccinated via the same route with the same dose of ChAdOx1 GFP (one animal 56 and 28 days before challenge, five animals 28 days before challenge, Figure 2a). No adverse events were observed upon vaccination. Spike-specific antibodies were present as early as 14 days post vaccination and were significantly boosted upon the second immunization (two-tailed Wilcoxon test). Endpoint IgG titers of 400-6400 (prime) and 400-19,200 (prime-boost) were measured on the day of challenge (Figure 2b). Virus-specific neutralising antibodies were also significantly boosted upon secondary immunization (two-tailed Wilcoxon test) and detectable in all vaccinated animals before challenge (5-40 (prime) and 10-160 (prime-boost)), whereas no virus-specific neutralising antibodies were detected in control animals (Figure 2c). IgM antibodies were present in serum post vaccination at challenge in 6 out of 6 prime-boost and 2 out of 6 prime only animals (Extended Data Figure 2b). SARS-CoV-2 spike specific T-cell responses were detected at 0 DPI by IFN-γ ELISpot assay upon stimulation of peripheral blood mononuclear cells (PBMCs) with a peptide library spanning the full length of the spike protein. No statistically significant difference in the magnitude of the response was found between the prime-boost and prime-only group (Mann Whitney test, p-value = 0.3723, Figure 2d). As reported previously[6], vaccination with ChAdOx1 nCoV-19 resulted in the induction of neutralizing antibodies against the vaccine vector itself within 28 days after vaccination (Extended Data Figure 3). Nonetheless, a boost vaccination with ChAdOx1 nCoV-19 resulted in a significant increase in binding and neutralizing antibodies in NHPs (Figure 2b,c) and increase of SARS-CoV-2 VN titer was not significantly correlated to ChAdOx1 VN titer (two-tailed Pearson correlation, r^2=0.6493 p=0.0529).

Clinical signs

Upon challenge with 2.6×10^6 TCID$_{50}$ SARS-CoV-2 to both the upper and lower respiratory tract the average clinical score of control animals was higher compared to ChAdOx1 nCoV-19 vaccinated animals. This was significantly different as determined via Mann-Whitney's rank's test on 4-7 days post infection (DPI, Figure 3a).

Viral load in respiratory tract samples

In BAL fluid obtained from control animals, viral genomic RNA (gRNA) and subgenomic RNA (sgRNA), indicative of virus replication, was detected on all days. In contrast, viral gRNA and sgRNA was detected in only two vaccinated animals on 3 DPI, and viral load was significantly lower (Figure 3b). Viral gRNA was detected in nose swabs from all animals and no difference was found on any day between vaccinated and control animals. Viral sgRNA was detected in a minority of animals, with no difference between groups (Figure 3c). Infectious virus could only be detected at 1 and 3 DPI in prime-only vaccinated and control animals, and 1 DPI in prime-boost vaccinated animals (Extended Data Table 1).

Cytokine response

Cytokines in serum were analysed after challenge to monitor immune responses. Following vaccination of NHPs the level of Th1 (IFN-γ and IL-2) or Th2 (IL-4, IL-5, or IL-13) cytokines in the serum were low and no evidence of a dominant Th2 response was detected (Extended Data Figure 4). We observed a significant upregulation in IFN-γ at 1 DPI in ChAdOx1 nCoV-19 prime-only vaccinated animals, but not in prime-boost vaccinated or control animals. IL-10 and IL-13 were significantly upregulated in control animals compared to prime-boost animals on 1 and 7 DPI (IL-13 only), but not compared to prime-only animals. No significant differences were observed between ChAdOx1 nCoV-19 and control animals for TNF-α, IL-2, IL-4, IL-5 and IL-6 (Extended Data Figure 4); this is in line with a previously observed lack of upregulation

of cytokines and chemokines in rhesus macaques upon infection with SARS-CoV-2.

Pulmonary pathology and viral load

At 7 days post inoculation, all animals were euthanized, and tissues were collected. None of the vaccinated monkeys developed pulmonary pathology after inoculation with SARS-CoV-2. All lungs were histologically normal and no evidence of viral pneumonia nor immune-enhanced inflammatory disease was observed. In addition, no SARS-CoV-2 antigen was detected by immunohistochemistry in the lungs of any of the vaccinated animals. Three out of 6 control animals developed some degree of viral interstitial pneumonia. Lesions were widely separated and characterized by thickening of alveolar septae by small amounts of edema fluid and few macrophages and lymphocytes. Alveoli contained small numbers of pulmonary macrophages and, rarely, edema. Type II pneumocyte hyperplasia was observed. Multifocally, perivascular infiltrates of small numbers of lymphocytes forming perivascular cuffs were observed. Immunohistochemistry demonstrated viral antigen in type I and II pneumocytes, as well as in alveolar macrophages, in 5 out of 6 control animals (Figure 4). We were unable to detect any lesions or IHC-positive cells in nasal mucosa in any animals.

Viral gRNA load was high in lung tissue of all control animals and viral sgRNA was detected in 5 out of 6 control animals (Figure 3d). In the prime-only vaccinated group, the viral gRNA load was significantly lower in lung tissue as determined via Mann-Whitney's rank test, and below limit of detection in 2 of 6 vaccinated animals; viral sgRNA was only detected in lung tissue of one animal (Figure 3d, Extended Data Figure 5). In the prime-boost vaccinated group, viral gRNA was detected in 2 out of 6 animals; one animal was weakly positive in one lung lobe and one animal which mounted a limited response to vaccination was positive in four lung lobes. Viral sgRNA could only be detected in lung tissue from the animal with a lower immune response (Figure 3d, Extended Data Figure 5). We did not detect infectious virus in any lung tissue.

Extra-pulmonary pathology and viral load

No lesions were observed in gastro-intestinal tissues of any animals. As reported previously[7], SARS-CoV-2 antigen could be detected in lymphocytes and macrophages in the lamina propria of the intestinal tract of all control animals. This phenomenon was also observed in 6 out of 6 prime vaccinated animals and 3 out of 6 prime-boost vaccinated animals. There were no histological differences between lymphoid tissues of vaccinated or control animals.

Viral gRNA could be detected in extra-respiratory tissues but was predominantly low in all animals and not associated with detection of sgRNA. Viral gRNA in intestinal tissues of prime-boost vaccinated animals was higher than that measured in control and prime-only vaccinated animals and was associated with the detection of sgRNA. However, no infection of intestinal tissue was observed by immunohistochemistry, nor were we able to detect infectious virus in intestinal tissue (Extended Data Figure 6).

Discussion

Here, we show that a single vaccination with ChAdOx1 nCoV-19 is effective in preventing damage to the lungs upon high dose challenge to both upper and lower respiratory tract with SARS-CoV-2, and a prime-boost regimen significantly increased humoral immune responses. We did not see an increase in cellular responses following a second dose of ChAdOx1 nCoV-19, in line with previous results reported by Bliss *et al.* using a homologous prime-boost regimen with malaria vaccine candidate ChAd63 ME-TRAP[8]. A small decrease in S and vector-specific antibody titers was observed between D-14 and D0 in the prime-boost

group. Longitudinal preclinical and clinical studies will investigate whether this decline in antibody titer is significant over time.

Two recently published SARS-CoV-2 vaccine studies in NHPs showed similar results: Gao et al. used a three dose vaccination regimen of a high dose of whole inactivated SARS-CoV-2 which protected rhesus macaques from SARS-CoV-2 pneumonia[9] and Yu et al. used a two dose vaccination regimen with a DNA vaccine encoding spike protein which significantly reduced viral RNA presence in BAL fluid and nasal swabs[10]. The three studies were conducted at different locations using different protocols, and thus direct comparison is difficult. Animals were challenged with 1×10^6 TCID$_{50}$ by the intratracheal route[9], 1.1×10^4 PFU via the intratracheal and intranasal route[10], or here, animals were challenged with 2.6×10^6 TCID$_{50}$ via the intratracheal, intranasal, ocular and oral route. VN titers induced in vaccinated animals were similar between studies; vaccination with two doses of ChAdOx1 nCoV-19, three doses of 6 μg inactivated SARS-CoV-2, or two doses of spike-encoding DNA resulted in median VN titres of 80, 50, and 74, respectively. A prime vaccination with ChAdOx1 nCoV-19 resulted in VN titers similar to those obtained after inoculation of rhesus macaques with SARS-CoV-2[7]. Upon vaccination with one or two doses of ChAdOx1 nCoV-19, viral load in BAL fluid and lung tissue of vaccinated animals was significantly reduced, suggesting that vaccination prevents or strongly reduces virus replication in the lower respiratory tract. Despite this marked difference in virus replication in the lungs, we did not observe reduction in viral shedding from the nose in either the prime-only or prime-boost regimen. Interestingly, viral RNA in nose swabs from vaccinated animals was also detected by Yu et al[10], whereas Gao et al[9] challenged only the lower respiratory tract with SARS-CoV-2 and did not assess nasal shedding.

Our primary goal for a vaccine against SARS-CoV-2 is to prevent disease, and we did not observe pneumonia or viral antigen in the lungs of vaccinated animals. Based on the data presented here, it is possible that a single or double dose of ChAdOx1 nCoV-19 will not prevent infection nor transmission of SARS-CoV-2. However, it could significantly reduce illness. Animals in this study were challenged with a high dose of virus via multiple routes as a stringent test of the protective efficacy of the vaccine and absence of enhanced disease upon infection. This does not reflect a realistic human exposure regarding route and dose. Future studies will determine whether changing the route of vaccination to expose mucosal surfaces will induce mucosal immunity, which may result in reduced nasal shedding and onward transmission. It should be noted that detection of sgRNA in nasal swabs was low with lower levels also detected in intestinal tissues. No viral antigen could be detected by immunohistochemistry, and it is not yet clear whether virus is replicating in the nasal mucosa of vaccinated animals.

Several preclinical studies of vaccines against SARS-CoV resulted in immunopathology after vaccination and subsequent challenge, with more severe disease in vaccinated animals than in controls[11–13]. Immune-enhanced disease could be observed as early as 2 days post challenge in mice[14,15] and 7 days post challenge in NHPs[13]. Immune-enhanced disease was associated with a Th2 response in mice[14] and diffuse alveolar damage and cellular infiltrates in NHPs[13]. In this study, there was no evidence of immune-enhanced disease in vaccinated animals. The immune response was not skewed towards a Th2 response in mice or NHPs, there was no increase in clinical signs or virus replication throughout the study in vaccinated NHPs compared to controls and no markers of disease enhancement in pulmonary tissue of NHPs such as an influx of cells or diffuse alveolar damage were observed. Clinical scoring of vaccinated animals was lower than clinical scoring in control animals, again strongly suggesting absence of immune-enhanced disease.

Results from ongoing clinical trials will be the most informative in determining whether ChAdOx1 nCoV-19 will be an appropriate vaccine candidate, but the results presented in the current study are encouraging. The data presented here informed the start of the phase I clinical trial with ChAdOx1 nCoV-19 on April 23, 2020. As of July 1st, 2020, more than 8000 volunteers have participated in the randomised controlled clinical trials. This study is thus an important step towards the development of a safe and efficacious SARS-CoV-2 vaccine.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41586-020-2608-y.

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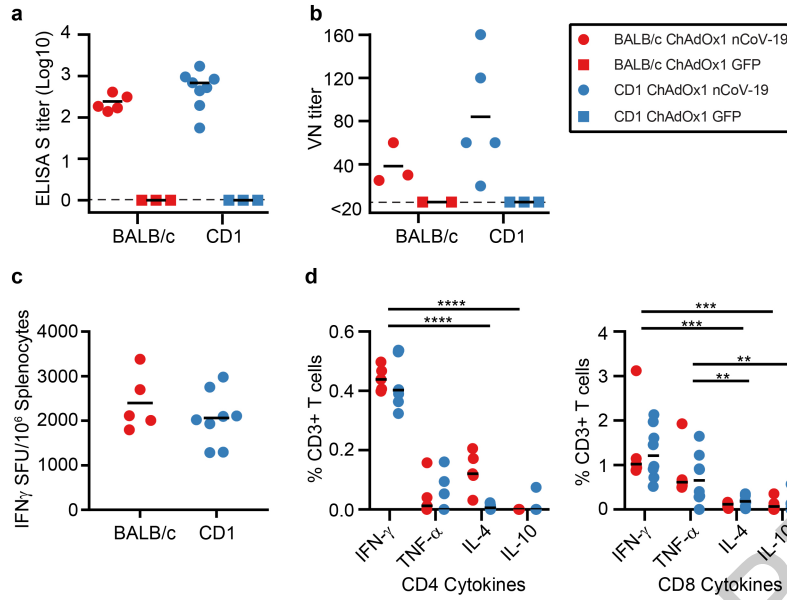
Fig. 1 | Humoral and cellular immune responses to ChAdOx1 nCoV-19 vaccination in mice. a. End point titer of serum IgG detected against S protein at 14 days post vaccination. No positive responses were detected in the control group. n=5, 3, 8, and 3 animals respectively examined over 1 independent experiment. b. Virus neutralizing titer in serum at 9 days post vaccination. n=3, 2, 5, and 3 animals respectively examined over 1 independent experiment. c. Summed IFN-γ ELISpot responses in splenocytes toward peptides spanning the spike protein at 14 days post vaccination. Control mice had low (<100 SFU) responses. n=5 and 8 animals respectively examined over 1 independent experiment. d. Summed frequency of spike-specific cytokine positive CD3+ T cells at 14 days post vaccination. P-value left panel: <0.0001. P-value right panel 0.0002 (IFN-γ−IL-4); 0.0001 (IFN-γ-IL-10); 0.0054 (TNF-α-IL-4); 0.0022 (TNF-α-IL-10). n=5 and 8 animals respectively examined over 1 independent experiment. BALB/c = red; CD1 = blue; vaccinated = circle; control = square; dotted line = limit of detection; line = mean; SFU = spot-forming units; Spl. = splenocytes; * = p<0.05. Statistical significance determined via 2-way ANOVA (repeated measure) and post-hoc positive test.
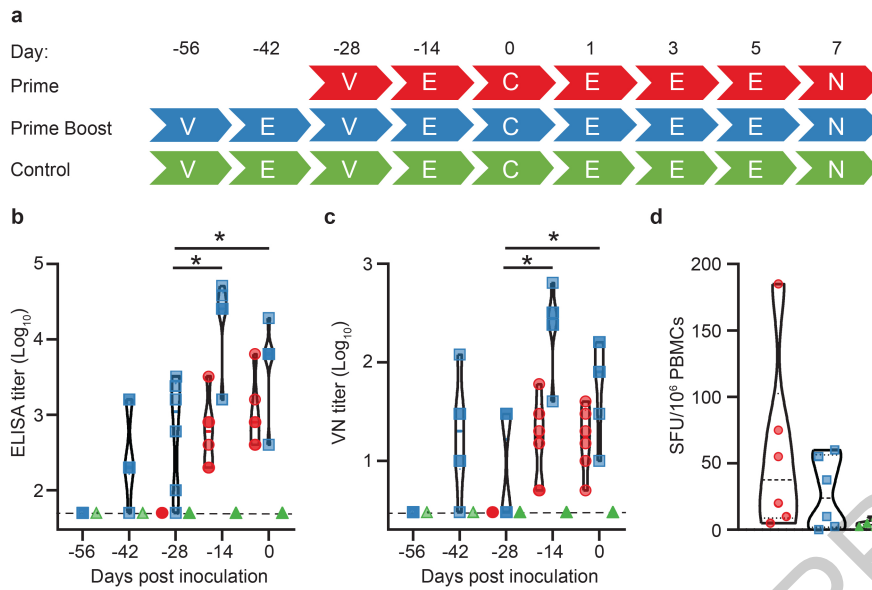
Fig. 2 | Humoral and cellular immune responses to ChAdOx1 nCoV-19 vaccination in rhesus macaques. a. Study schedule for NHPs. V = vaccination; E = exam; C = exam and challenge; N = exam and necropsy. Violin plots of b. endpoint IgG titer in serum against trimeric spike protein, c. VN titer in serum and d. Summed IFN-γ ELISpot responses in PBMCs collected at 0 DPI toward peptides spanning the spike protein. Red circles = prime-only vaccine; blue squares = prime-boost vaccine; green triangle = controls; dotted line = limit of detection; SFU = spot-forming units; VN= virus-neutralizing; * = p-value=0.0313. Statistical significance determined via two-tailed Wilcoxon test.
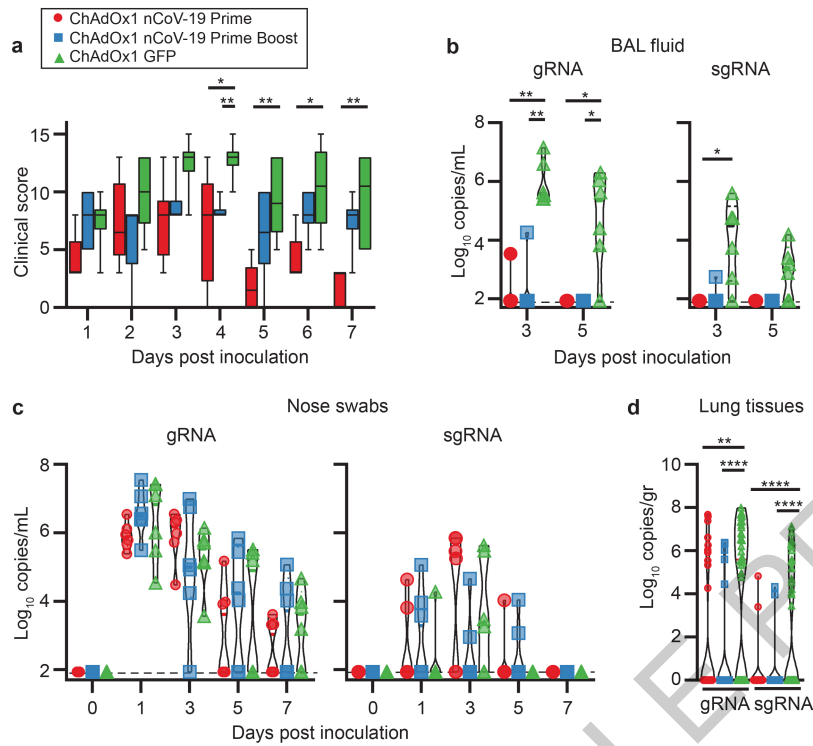
Fig. 3 | Clinical signs and viral load in rhesus macaques inoculated with SARS-CoV-2 after vaccination with ChAdOx1 nCoV-19. a. Boxplot of 25th to 75th percentile with median as centre and whiskers of 5th to 95th percentile clinical score in NHPs. n=6 animals per group examined over 2 independent experiments. P-values = 0.0455 (Prime-control D3); 0.0238 (Prime-control D4); 0.0043 (Prime-boost-control D4); 0.0043 (Prime-control D5); 0.0152 (Prime-control D6); 0.0022 (Prime-control D7). Violin plot of viral load in b. BAL fluid (* = p-value 0.0152; ** = p-value 0.0022) and c. nose swabs obtained from rhesus macaques. d. Violin plot of viral load in lung tissue. n=6 lung lobes of 6 animals per group examined over 2 independent experiments. **=p-value=0.0011; ****=p-value<0.0001. Dotted line = limit of detection. Statistical significance determined via two-tailed Mann-Whitney test.
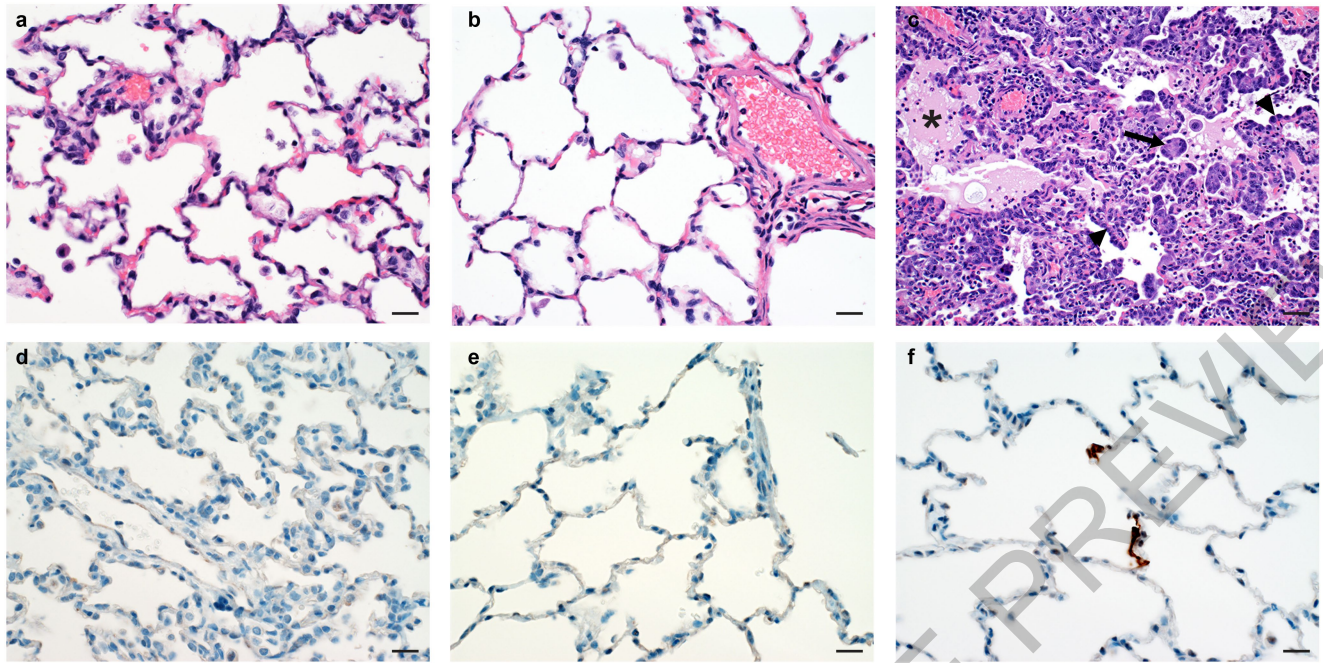
Fig. 4 | Histological changes in lungs of rhesus macaques on 7 DPI. No histological changes were observed in the lungs of ChAdOx1 nCoV-19 prime (a) and prime-boost (b) vaccinated animals. c) Interstitial pneumonia with edema (asterisk), type II pneumocyte hyperplasia (arrowhead) and syncytial cells (arrow) in control animals. No SARS-CoV-2 antigen was detected by immunohistochemistry in the lungs of ChAdOx1 nCoV-19 prime (d) and prime-boost (e) vaccinated animals. f) SARS-CoV-2 antigen (visible as red-brown staining) was detected by immunohistochemistry in type I and type II pneumocytes in the lungs of control animals. 18 sections, taken from 6 different lung lobes are evaluated for each animal; a representative lesion from each group was selected for the figure. Magnification: 400x, scale bar = 20 μm.

Methods

Ethics Statement

Mice. Mice were used in accordance with the UK Animals (Scientific Procedures) Act under project license number P9804B4F1 granted by the UK Home Office. Animals were group housed in IVCs under SPF conditions, with constant temperature (20–24 °C) and humidity (45 to 65%) with lighting on a fixed light/dark cycle (12-hours/12-hours)

NHPs. Animal experiment approval was provided by the Institutional Animal Care and Use Committee (IACUC) at Rocky Mountain Laboratories. Animal experiments were executed in an Association for Assessment and Accreditation of Laboratory Animal Care (AALAC)-approved facility by certified staff, following the basic principles and guidelines in the NIH Guide for the Care and Use of Laboratory Animals, the Animal Welfare Act, United States Department of Agriculture and the United States Public Health Service Policy on Humane Care and Use of Laboratory Animals. Rhesus macaques were housed in individual primate cages allowing social interactions, in a climate-controlled room with a fixed light/dark cycle (12-hours/12-hours) and monitored a minimum of twice daily. Commercial monkey chow, treats, and fruit were provided by trained personnel. Water was available ad libitum. Environmental enrichment consisted of a variety of human interaction, commercial toys, videos, and music. The Institutional Biosafety Committee (IBC) approved work with infectious SARS-CoV-2 virus strains under BSL3 conditions. All sample inactivation was performed according to IBC approved standard operating procedures for removal of specimens from high containment.

Generation of vaccine ChAdOx1 nCoV-19

The spike protein of SARS-CoV-2 (GenBank accession number YP_009724390.1), the surface glycoprotein responsible for receptor binding and fusion/entry into the host cell, was codon optimised for expression in human cell lines and synthesised with the tissue plasminogen activator (tPA) leader sequence at the 5' end by GeneArt Gene Synthesis (Thermo Fisher Scientific). The sequence, encoding SARS-CoV-2 amino acids 2-1273 and tPA leader, was cloned into a shuttle plasmid using InFusion cloning (Clontech). The shuttle plasmid encodes a modified human cytomegalovirus major immediate early promoter (IE CMV) with tetracycline operator (TetO) sites, poly adenylation signal from bovine growth hormone (BGH), between Gateway® recombination cloning sites. ChAdOx1 nCoV-19 was prepared using Gateway® recombination technology (Thermo Fisher Scientific) between the shuttle plasmid described and the ChAdOx1 destination DNA BAC vector described in[5] resulting in the insertion of the SARS-CoV-2 expression cassette at the E1 locus. The ChAdOx1 adenovirus genome was excised from the BAC using unique PmeI sites flanking the adenovirus genome sequence. The virus was rescued and propagated in T-Rex 293 HEK cells (Invitrogen) which repress antigen expression during virus propagation. Purification was by CsCl gradient ultracentrifugation. Virus titers were determined by hexon immunostaining assay and viral particles calculated based on spectrophotometry[16,17].

Study design animal experiments

Mice. Female BALB/cOlaHsd (BALB/c) (Envigo) and outbred Crl:CD1 (ICR) (CD1) (Charles River) mice of at least 6 weeks of age, were immunized IM in the musculus tibialis with 6×10^9 VP of ChAdOx1 nCoV-19 unless otherwise stated.

NHPs. 18 adult rhesus macaques (17M, 1F) between 2-4 years old were randomly divided into three groups of six animals. Animal group size was based on initial model development. Group 1 was vaccinated with ChAdOx1 nCoV-19 at -28 DPI, group 2 was vaccinated with ChAdOx1 nCoV-19 at -56 and -28 DPI, group 3 was vaccinated with ChAdOx1 GFP at -56 and -28 DPI (1 animal) or at -28 DPI (5 animals). All vaccinations were done with 2.5×10^{10} VP/animal diluted in sterile PBS. Blood samples were obtained before vaccination and 14 days thereafter. Animals were challenged with SARS-CoV-2 strain nCoV-WA1-2020 (MN985325.1) diluted in sterile DMEM on 0 DPI; with administration of 4 mL intratracheally, 1 mL intranasally, 1 mL orally and 0.5 mL ocularly of 4×10^5 $TCID_{50}$/mL virus suspension. Animals were scored daily by the same person who was blinded to study group allocations using a standardized scoring sheet. Scoring was based on the following criteria: general appearance, appearance skin and coat, discharge, respiration, feces and urine appearance, appetite, and activity. The scoring sheet can be found in[7]. Clinical exams were performed on -28, -14, 0, 1, 3, and 5 and 7 DPI. Nasal swabs and blood were collected at all exam dates. BAL was performed on 3, 5, and 7 DPI by insertion of an endotracheal tube and bronchoscope into the trachea, then past the 3rd bifurcation, and subsequent installation of 10 mL of sterile saline. Manual suction was applied to retrieve the BAL sample. Serum biochemistry (albumin, AST, ALT, GGT, BUN, creatinine) was analyzed using the Vetscan VS2 Chemistry Analyzer and VetScan 2 Preventative Care 12 discs (Abaxis) (Tables S1–3). Necropsy was performed on 7 DPI and the following tissues were collected: cervical lymph node, mediastinal lymph node, conjunctiva, nasal mucosa, oropharynx, tonsil, trachea, all six lung lobes, right and left bronchus, heart, liver, spleen, kidney, stomach, duodenum, jejunum, ileum, cecum, colon, urinary bladder.

Cells and virus

SARS-CoV-2 strain nCoV-WA1-2020 (MN985325.1) was provided by CDC, Atlanta, USA. Virus propagation was performed in VeroE6 cells in DMEM supplemented with 2% fetal bovine serum, 1 mM L-glutamine, 50 U/ml penicillin and 50 µg/ml streptomycin. The used virus stock was 100% identical to the initial deposited genbank sequence (MN985325.1) and no contaminants were detected. VeroE6 cells were maintained in DMEM supplemented with 10% fetal bovine serum, 1 mM L-glutamine, 50 U/ml penicillin and 50 µg/ml streptomycin. VeroE6 cells were provided by Dr. Ralph Baric and were not authenticated in-house; mycoplasma testing is performed at regular intervals and no mycoplasma has been detected.

Virus isolation from tissue

Tissue sections were weighed and homogenized in 1 mL of DMEM. 250 µl of homogenate was added to VeroE6 cells in a 24 well plate in duplicate. After 1 hour at 37 °C and 5% CO_2, cells were washed with PBS and 500 µl of DMEM containing 2% FBS was added. Cells were incubated at 37 °C and 5% CO_2. CPE was read 6 days later.

Virus neutralization assay SARS-CoV-2

Sera were heat-inactivated (30 min, 56 °C), two-fold serial dilutions were prepared in 2% DMEM and 100 $TCID_{50}$ of SARS-CoV-2 was added. After 1hr incubation at 37 °C and 5% CO2, virus:serum mixture was added to VeroE6 cells and incubated at 37 °C and 5% CO2. At 5 dpi, cytopathic effect was scored. The virus neutralization titer was expressed as the reciprocal value of the highest dilution of the serum which still inhibited virus replication.

Virus neutralization assay ChAdOx1

Chimpanzee adenovirus ChAdOx1-specific neutralizing antibody titers were assessed using a secreted placental alkaline phosphatase (SEAP) quantitation assay as described[6]. Briefly, GripTite MSR 293 cells (Invitrogen, catalog no. R795-07) were infected with the serial diluted serum in phenol red–free DMEM (Life Technologies, catalog no. 31053028) and the ChAdOx1-SEAP reporter virus in a 1:1 mixture for 1 hour before replacing with phenol red–free 10% FBS DMEM for 24 hours. For each sample, SEAP concentration was assessed in 50 µl aliquots of culture supernatant, with CPSD as an indicator substrate (Tropix Phospha-Light Chemiluminescent Assay Kit, Life Technologies, catalog no. T1017). Luminescence intensity was measured using

a Varioskan Flash luminometer (Thermo Fisher Scientific). Serum dilution neutralization titers were measured by linear interpolation of adjacent values (to 50% inhibition) to determine the serum dilution required to reduce SEAP concentration by 50% compared to wells with virus alone.

RNA extraction and quantitative reverse-transcription polymerase chain reaction

Tissues (up to 30 mg) were homogenized in RLT buffer and RNA was extracted using the RNeasy kit (Qiagen) according to the manufacturer's instructions. RNA was extracted from BAL fluid and nasal swabs using the QiaAmp Viral RNA kit (Qiagen) according to the manufacturer's instructions. Viral gRNA[18] and sgRNA[19] specific assays were used for the detection of viral RNA. Five µl RNA was tested with the Rotor-GeneTM probe kit (Qiagen) according to instructions of the manufacturer. Dilutions of SARS-CoV-2 standards with known genome copies were run in parallel.

Enzyme-linked immunosorbent assay for mouse sera

MaxiSorp plates (Nunc) were coated with S1 (monomeric, AA 1-674) or S2 (monomeric, AA 685-1211 The Native Antigen Company; 50 ng/well) or with prefusion stabilized SARS-CoV-2 spike protein (250 ng/well) in PBS for overnight adsorption at 4 °C. Plates were washed in PBS/Tween (0.05% v/v) and wells blocked using casein (ThermoFisher Scientific) for 1hr at RT. Serially diluted mouse serum samples were added and incubated overnight at 4 °C or 37 °C for 2hr for specific IgM detection. Plates were washed and Alkaline Phosphatase-conjugated goat anti-mouse IgG (Sigma) or IgM (Abcam) was added to all wells for 1hr at RT or 2hr at 37 °C, respectively. After washing pNPP substrate (Sigma) was added. Optical density (OD) values for each well were measured at 405 nm. Endpoint titers were calculated as follows: the $\log_{10}$ OD against $\log_{10}$ sample dilution was plotted and a regression analysis of the linear part of this curve allowed calculation of the endpoint titer with an OD of three times the background. The same calculation was used for diluting the sera to the same amounts of total IgG for further testing on different IgG subclasses with anti-mouse IgG subclass-specific antibodies (Abcam). The results of the IgG subclass ELISA are presented using OD values.

Enzyme-linked immunosorbent assay for NHP sera

Prefusion stabilized SARS-CoV-2 spike protein with a T4 fibritin trimerization motif[20] was obtained from the Vaccine Research Centre, Bethesda, USA. Maxisorp plates (Nunc) were coated overnight at 4 °C with 100 or 250 ng/well spike protein in PBS for IgG and IgM detection, respectively. Plates were blocked with 100 µl of casein in PBS (Thermo Fisher) for 1hr at RT. Serum serially diluted 2x in casein in PBS was incubated at RT for 1hr or 37 °C for 2hr. Antibodies were detected using affinity-purified polyclonal antibody peroxidase-labeled goat-anti-monkey IgG (Seracare, 074-11-021) in casein and TMB 2-component peroxidase substrate (Seracare, 5120-0047), developed for 5-10 min, and reaction was stopped using stop solution (Seracare, 5150-0021) and read at 450 nm or anti-monkey IgM – AP labelled after adding the pNPP substrate and measuring OD values at 405 nm. All wells were washed 4x with PBST 0.1% tween in between steps. Threshold for positivity was set at 3x OD value of negative control (serum obtained from non-human primates prior to start of the experiment) or 0.2, whichever one was higher, or OD of three times the background for the calculation of the IgM endpoint values.

ELISpot assay and ICS analysis

Single cell suspension of murine splenocytes were prepared by passing cells through 70µM cell strainers and ACK lysis prior to resuspension in complete media. Rhesus macaque PBMCs were isolated from ethylene diamine tetraaceticacid (EDTA) whole blood using LeucosepTM tubes (Greiner Bio-one International GmbH) and Histopaque®-1077 density gradient cell separation medium (Sigma-Aldrich) according to the manufacturers' instructions.

Mice. For analysis of IFN-γ production by ELISpot, cells were stimulated with pools of S1 or S2 peptides (final concentration of 2µg/ml) on IPVH-membrane plates (Millipore) coated with 5µg/ml anti-mouse IFN-γ (AN18). After 18-20 hours of stimulation, IFN-γ spot forming cells (SFC) were detected by staining membranes with anti-mouse IFN-γ biotin (1µg/ml) (R46A2) followed by streptavidin-Alkaline Phosphatase (1µg/ml) and development with AP conjugate substrate kit (BioRad, UK).

For analysis of intracellular cytokine production, cells were stimulated at 37°C for 6 hours with 2µg/ml S1 or S2 pools of peptide, media or cell stimulation cocktail (containing PMA-Ionomycin, Biolegend), together with 1µg/ml Golgi-plug (BD) with the addition of 2µl/ml CD107a-Alexa647. Cell supernatant was collected and frozen at −20°C for subsequent analysis by MesoScale Discovery (MSD) assay (see below). Following surface staining with CD4-BUV496, CD8-PerCPCy5.5, CD62L-BV711, CD127-BV650 and live-dead Aqua, cells were fixed with 4% paraformaldehyde and stained intracellularly with TNF-α-A488, IL-2-PECy7, IL-4-BV605, IL-10-PE and IFN-γ-e450 diluted in Perm-Wash buffer (BD).

Sample acquisition was performed on a Fortessa (BD) and data analyzed in FlowJo V10 (TreeStar). An acquisition threshold was set at a minimum of 5000 events in the live CD3+ gate. Antigen specific T cells were identified by gating on LIVE/DEAD negative, doublet negative (FSC-H vs FSC-A), size (FSC-H vs SSC), CD3+, CD4+ or CD8+ cells and cytokine positive (Gating strategy detailed in Supplementary Figure 1). Cytokine positive responses are presented after subtraction of the background response detected in the corresponding unstimulated sample (media containing CD107a and Golgi-plug) of each individual spleen sample. To determine whether there was a statistically significant differences in the level of cytokines produced by CD4+ or CD8+ T cells, data in each graph was analysed with a 2-way ANOVA (repeated measure) and post-hoc Tukey's multiple comparison test.

NHPs. IFN-γ ELISpot assay of PBMCs was performed using the ImmunoSpot® Human IFN- γ Single-Color Enzymatic ELISpot Assay Kit according to the manufacturer's protocol (Cellular Technology Limited). PBMCs were plated at a concentration of 100,000 cells per well and were stimulated with four contiguous peptide pools spanning the length of the SARS-CoV-2 spike protein sequence at a concentration of 2 µg/mL per peptide (Mimotopes). ELISpot plates were subjected to overnight formalin inactivation prior to removal from BSL4 for reading. Analysis was performed using the CTL ImmunoSpot® Analyzer and ImmunoSpot® Software (Cellular Technology Limited). Spot forming units (SFU) per 1.0×10^6 PBMCs were summed across the 4 peptide pools for each animal.

Measurement of cytokines and chemokines

Mouse samples were assayed using MSD Technology V-PLEX Mouse Cytokine 29-Plex kit according to the manufacturer's instructions. Non-human primate samples were inactivated with γ-radiation (2 MRad) according to standard operating procedures and assayed on a Bio-Plex 200 instrument (Bio-Rad) using the Non-Human Primate Cytokine MILLIPLEX map 23-plex kit (Millipore) according to the manufacturer's instructions. LLOD was used for all undetectable and extrapolated values. Only data for cytokines consistently above the lower limit of quantification were included in further analyses.

$\log_{10}$ Fold Change ($\log_{10}$FC) for mouse samples was calculated as follows: $\log_{10}FC = \log_{10}((\text{Stimulated (pg/ml)} + 1) / (\text{Unstimulated (pg/ml)} + 1))$

Fold change for NHP samples was calculated as follows: FC = Concentration (pg/mL) on DX (1, 3, 5, or 7)/Concentration (pg/mL) on D0

Article

Histology and immunohistochemistry

Necropsies and tissue sampling were performed according to IBC-approved protocols. Lungs were perfused with 10% formalin and processed for histologic review. Harvested tissues were fixed for eight days in 10% neutral-buffered formalin, embedded in paraffin, processed using a VIP-6 Tissue Tek (Sakura Finetek, USA) tissue processor, and embedded in Ultraffin paraffin polymer (Cancer Diagnostics, Durham, NC). Samples were sectioned at 5 µm, and resulting slides were stained with hematoxylin and eosin. Specific anti-CoV immunoreactivity was detected using an in-house SARS-CoV-2 nucleocapsid protein rabbit antibody (Genscript) at a 1:1000 dilution. The IHC assay was carried out on a Discovery ULTRA automated staining instrument (Roche Tissue Diagnostics) with a Discovery ChromoMap DAB (Ventana Medical Systems) kit. All tissue slides were evaluated by a board-certified veterinary anatomic pathologist blinded to study group allocations. 18 sections, taken from 6 different lung lobes are evaluated for each animal; a representative lesion from each group was selected for the figure.

Statistical analyses

Two-tailed Mann-Whitney's rank or Wilcoxon tests were conducted to compare differences between groups using Graphpad Prism version 8.3.0. A Bonferroni correction was used to control for type I error rate where required.

Reporting summary

Further information on research design is available in the Nature Research Reporting Summary linked to this paper.

Data availability

Data have been deposited in Figshare at https://figshare.com/articles/dataset/Figshare_document_xlsx/12290696.

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Extended Data Fig. 1 | Antigen specific responses following ChAdOx1 nCov19 vaccination. a. IgG subclass antibodies detected against S1 or S2 protein in sera of BALB/c or CD1 mice. b. Frequency of cytokine positive CD3+ T cells following stimulation of splenocytes with S1 pool (dark) or S2 pool (transparent) peptides in BALB/c (red) and CD1 (blue) mice. c. Log10 fold change in cytokine levels in supernatant from S1 (dark) and S2 (transparent) stimulated splenocytes when compared to corresponding unstimulated splenocyte sample for BALB/c and CD1 mice. n=5 (BALB/c) and 8 (CD1) animals examined over 1 independent experiment for all figure panels.

Extended Data Fig. 2 | Spike-specific serum IgM. a. Spike-specific serum IgM in mice 14 days post vaccination. n=5 (BALB/c) and 8 (CD1) animals examined over 1 independent experiment. b. Spike-specific serum IgM in NHPs upon prime-boost or prime-only vaccination. n=6 animals per group examined over 2 independent experiments.
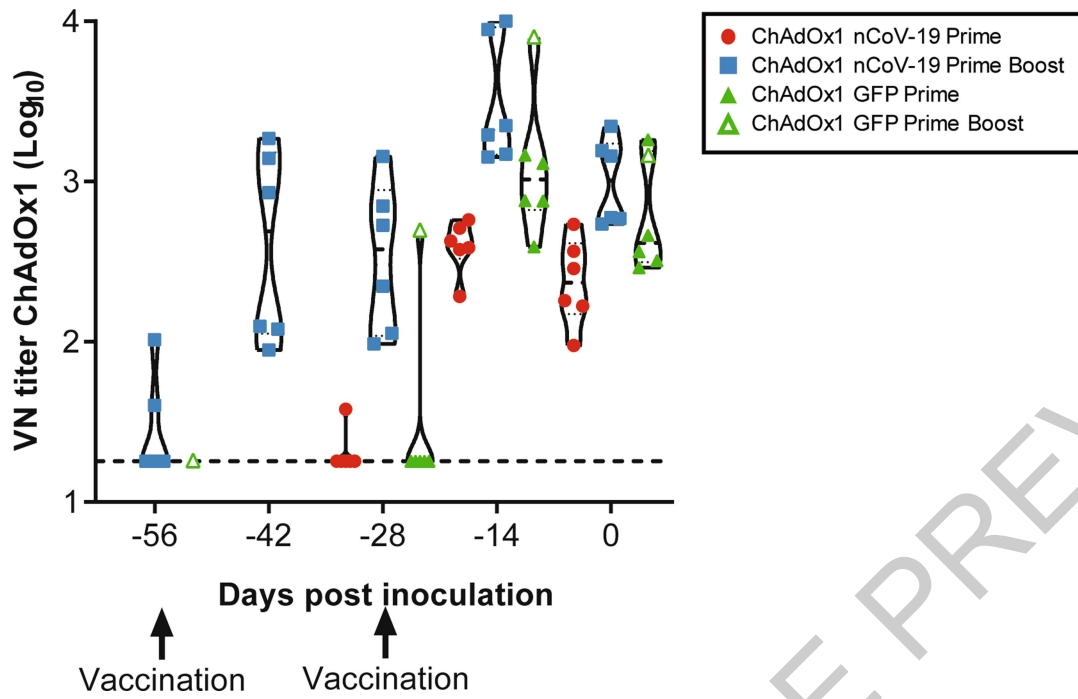
Extended Data Fig. 3 | ChAdOx1 neutralizing antibodies in serum of vaccinated NHPs. Control animal with prime-boost regimen is highlighted with open triangle symbol. VN = virus neutralizing. n=6 animals per group examined over 2 independent experiments.
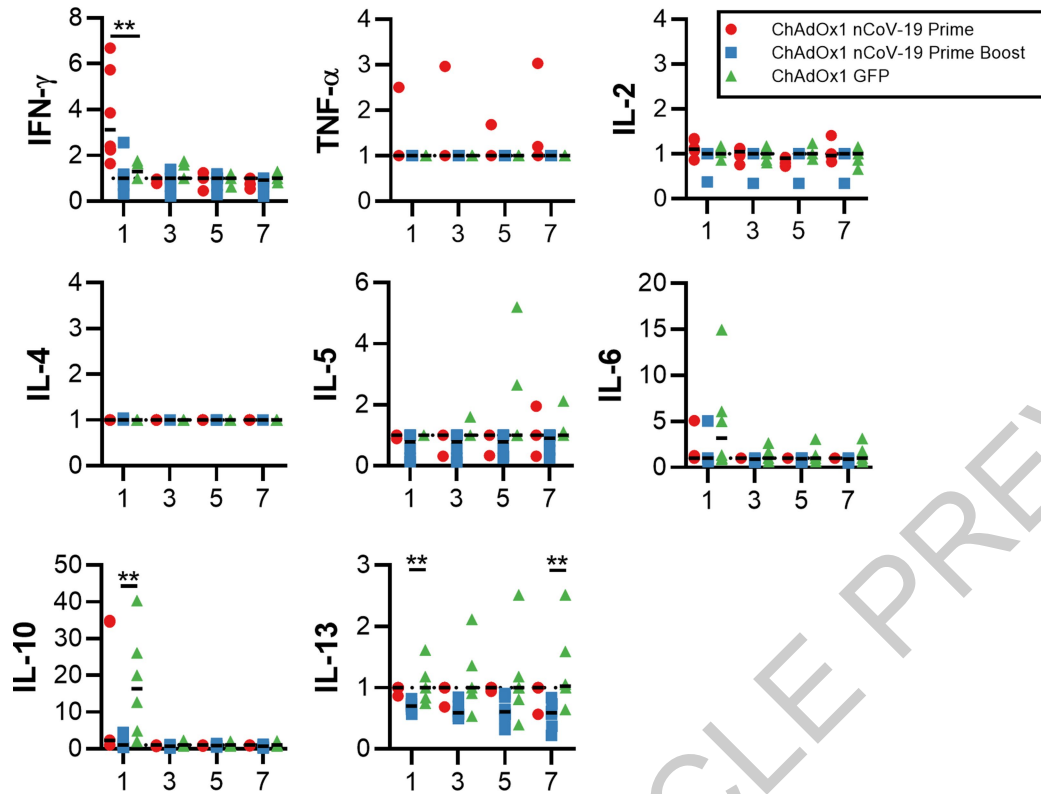
Extended Data Fig. 4 | Serum cytokines in rhesus macaques challenged with SARS-CoV-2. Fold increase in cytokines in serum compared to pre-challenge values. ** = p-value<0.01; Line = median. Statistical significance determined via two-tailed Mann-Whitney test. P-values: IFN-γ = 0.0087; IL-10 = 0.0043; IL-13 = 0.0043 and 0.0065. n=6 animals per group examined over 2 independent experiments.
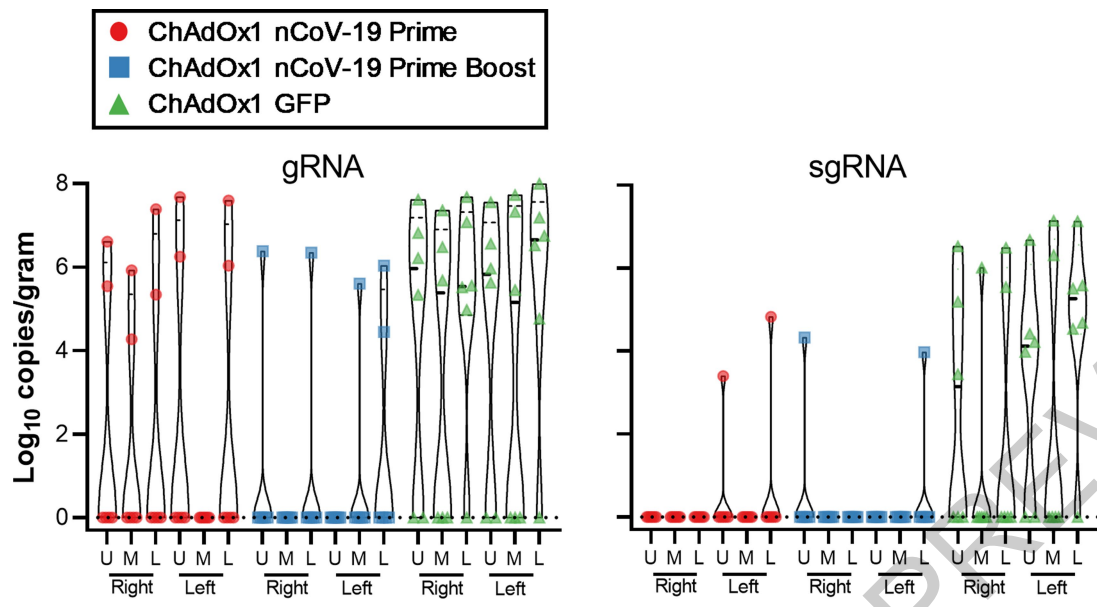
Extended Data Fig. 5 | Viral load in lung tissue of rhesus macaques challenged with SARS-CoV-2 at 7 DPI.
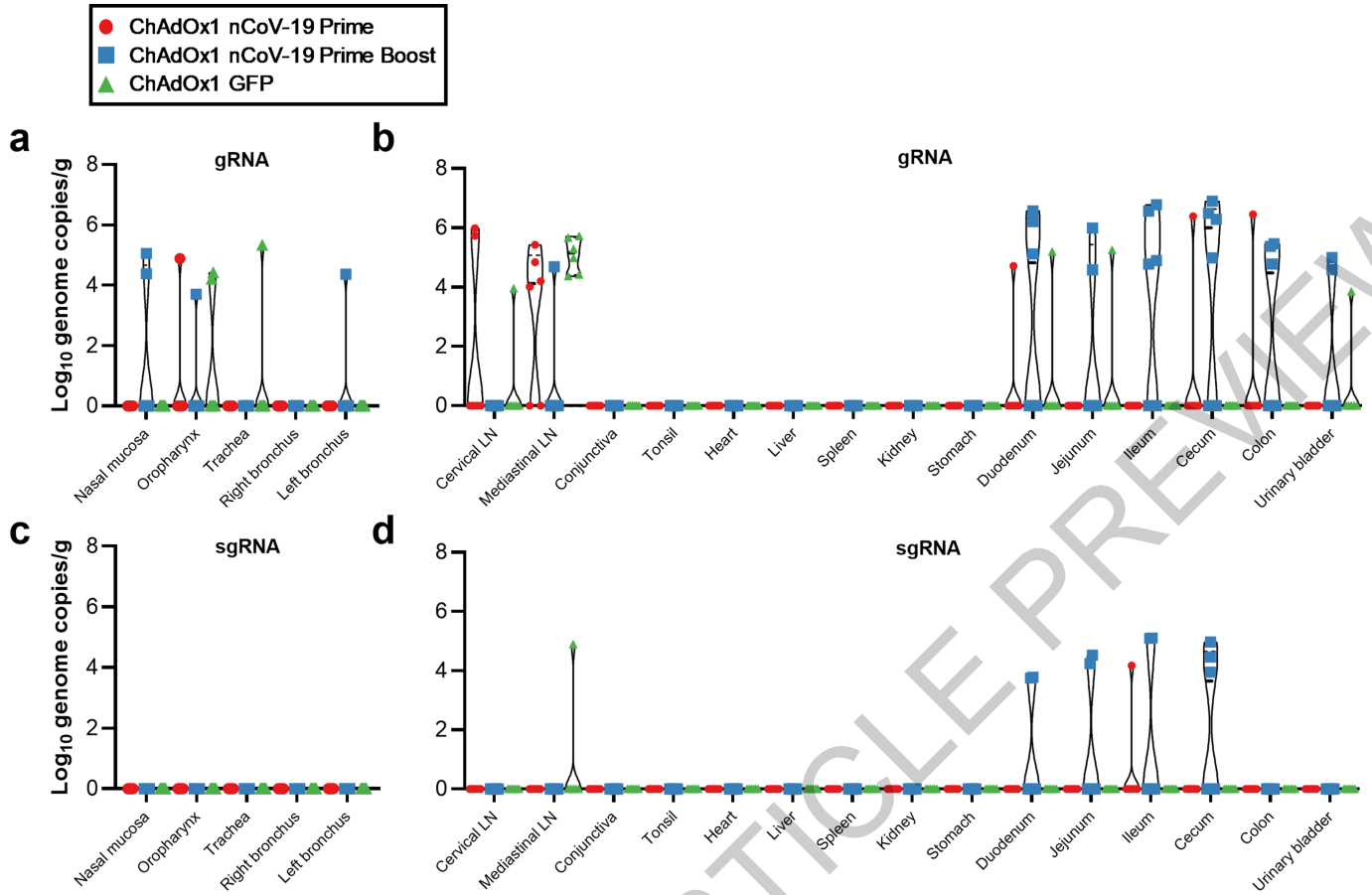
Extended Data Fig. 6 | Viral load in tissues of rhesus macaques challenged with SARS-CoV-2 at 7 DPI. a. Viral gRNA in respiratory tissues excluding lung tissue. b. Viral gRNA in non-respiratory tissues. c. Viral sgRNA in respiratory tissues excluding lung tissue. d. Viral sgRNA in non-respiratory tissues.

Extended Data Table 1 | Virus isolation from nasal swabs obtained from rhesus macaques post challenge with SARS-CoV-2

	ChAdOx1 nCoV-19 Prime	ChAdOx1 nCoV-19 Prime-boost	ChAdOx1 GFP
1 DPI	4/6	2/6	4/6
3 DPI	2/6	0/6	1/6
5 DPI	0/6	0/6	0/6
7 DPI	0/6	0/6	0/6

nature research

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Last updated by author(s):	7/21/2020

Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see Authors & Referees and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☒	☐	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☒	☐	A description of all covariates tested
☐	☒	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	No software was used.
Data analysis	Data were analyzed using Graphpad Prism V8.3.0, FlowJo V10, and CTL ImmunoSpot® 7.0.11.0 Professional Analyzer DC

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:
- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

Data have been deposited in Figshare: 10.6084/m9.figshare.12290696

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	NHP - Since this is a model with no prior data, it was not possible to perform a power analysis. The sample size was based on experience with other nonhuman primate models of respiratory disease where the numbers used were sufficient for statistical analyses. Mice - Sample size was determined based on previous experience measuring the immunogenicity of vaccines in inbred and outbred mice where the numbers used were sufficient for statistical analyses.
Data exclusions	No data were excluded.
Replication	Lung histology: for each animal (n=12 (vaccinated) or 6 (control)), 3 sections were evaluated from all 6 lung lobes. Cytokine analysis: serum samples were analyzed in duplicate from each animal for each timepoint; n=12 (vaccinated) or 6 (control) Serological analysis: Serum samples were analyzed in duplicate from each animal for each timepoint; n=12 (vaccinated) or 6 (control). Mouse experiments were repeated twice All repeats were successful. qRT-PCR assays were performed with in-house validated standards. Flow cytometry and ELISpot assays were performed with positive controls (PMA/IONO stimulation).
Randomization	Animals were randomly assigned to groups
Blinding	Blinding was done for the following personnel: - Person scoring animals daily - Veterinary pathologists reviewing histology - Clinical veterinarians performing exams

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology
☐	☒ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☐	☒ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used	in-house SARS-2 rabbit sera, GenScript CD4-BUV496, BD, cat#557984, clone 500A2, dilution 1 in 100 CD8-PerCPCy5.5, BD, cat# 564667, clone GK1.5, dilution 1 in 100 CD62L-BV711, eBioscience/Thermofisher, cat# 45-0081-82, clone 53-6.7, dilution 1 in 200 CD127-BV650, BioLegend, cat# 103028, clone IM7, dilution 1 in 100 TNF-a-A488, BioLegend, cat# 104445, clone MEL-14, dilution 1 in 100 IL-2-PECy7, BioLegend, cat# 121610, clone 1D4B, dilution 1 in 1000 IL-4-BV605, BioLegend, cat# 135043, clone A7R34, dilution 1 in 100 IL-10-PE, eBioscience/Thermofisher, cat# 48-7311-82, clone XMG1.2, dilution 1 in 100 IFN-g-e450, eBioscience/Thermofisher, cat# 25-7021-82, clone JES6-5H4, dilution 1 in 100 anti-monkey IgG (gamma) antibody, peroxidase-labeled, Seracare, cat# 5220-0333/074-11-021, Lot# 10329492, dilution 1:2500 anti-monkey IgM antibody, peroxidase-labeled, Rockland, cat# 617-105-007, Lot# 27986, dilution 1:5000 anti-mouse IgM antibody, peroxidase-labeled, Abcam, cat# ab98672, Lot# GR325319-6, dilution 1:5000 Alkaline Phosphatase-conjugated goat anti-mouse IgG, Sigma, cat# A3562, Lot# SLBK6489v, dilution 1:5000
Validation	Validation of cross-reactivity of SARS-CoV to SARS-CoV-2 in IHC was done in-house by embedding SARS-CoV-2 infected Vero cells in histogel and producing and staining histology slides. All other antibodies validated by supplier: Monkey IgM: Assay by immunoelectrophoresis resulted in a single precipitin arc against anti-Alkaline Phosphatase (calf intestine), anti-Goat Serum, Monkey IgM and Monkey Serum. No reaction was observed against other Monkey heavy or light chain proteins. Mouse IgM: Minimal cross-reactivity Human, Rat Mouse IgG: Anti-Mouse IgG (whole molecule)-Alkaline Phosphatase antibody is specific for normal mouse serum and mouse IgG. In Ouchterlony double diffusion assays, the antibody reacts with mouse IgG1, IgG2a, IgG2b, IgG3, IgA, and IgM.

All other antibodies were validated for use with mouse samples by the supplier.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	VeroE6: Ralph Baric, University of North Carolina, Chapel Hill, USA (not commercial) GripTite 293 MSR cell line: ThermoFisher, Cat# R79507 T-Rex-293 cell line: ThermoFisher, Cat# R71007
Authentication	Not authenticated in-house.
Mycoplasma contamination	Mycoplasma testing confirmed negative at regular intervals.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used.

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals	Rhesus macaques, Chinese origin, adult (2-6 years), 17 males, 1 female Mice – Female BALB/cOlaHsd (BALB/c) (Envigo) and outbred Crl:CD1(ICR) (CD1) (Charles River) mice of at least 6 weeks of age
Wild animals	No wild animals were used.
Field-collected samples	No samples were collected in the field.
Ethics oversight	Mice - Mice were used in accordance with the UK Animals (Scientific Procedures) Act under project license number P9804B4F1 granted by the UK Home Office. NHP - All animal experiments were approved by the Institutional Animal Care and Use Committee of Rocky Mountain Laboratories, NIH and carried out by certified staff in an Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) International accredited facility, according to the institution's guidelines for animal use, following the guidelines and basic principles in the NIH Guide for the Care and Use of Laboratory Animals, the Animal Welfare Act, United States Department of Agriculture and the United States Public Health Service Policy on Humane Care and Use of Laboratory Animals.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Flow Cytometry

Plots

Confirm that:

☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☒ All plots are contour plots with outliers or pseudocolor plots.

☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	Sample preparation: Single cell suspension of murine splenocytes were prepared by passing cells through 70µM cell strainers and ACK lysis prior to resuspension in complete medium. Cells were stimulated at 37oC for 6 hours with 2µg/ml S1 or S2 pools of peptide, media or cell stimulation cocktail (containing PMA-Ionomycin, Biolegend), together with 1µg/ml Golgi-plug (BD) with the addition of 2µl/ml CD107a-Alexa
Instrument	BD Fortessa X2
Software	BD FACSDiva Software Version 8.0.2, FlowJo version 10 for analysis
Cell population abundance	An acquisition threshold was set at a minimum of 5000 events in the live CD3+ gate
Gating strategy	Antigen specific T cells were identified by gating on LIVE/DEAD negative, doublet negative (FSC-H vs FSC-A), size (FSC-H vs SSC), CD3+, CD4+ or CD8+ cells and cytokine positive. Cytokine positive responses are presented after subtraction of the background response detected in the corresponding unstimulated sample (media containing CD107a and Golgi-plug) of each individual spleen sample.

☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.