Development of an inactivated vaccine candidate for SARS-CoV-2

Qiang Gao1, Linlin Bao2, Haiyan Mao3, Lin Wang1, Kangwei Xu4, Minnan Yang5, Yajing Li1, Ling Zhu1, Nan Wang2, Zhe Lu3, Hong Gao2, Xiaoqin Ge2, Biao Kan2, Yaling Hu2, Jianqiu Liu2, Fang Cai2, Deyu Jiang1, Yanyun Yin1, Chengfeng Qin1, Jing Li1, Xuejie Gong1, Xiuyu Lou3, Wen Shi3, Xiuxiang Xiao4, Weihe Yin1, Yanjun Zhang3, Chuan Qin2

The coronavirus disease 2019 (COVID-19) pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has resulted in an unprecedented public health crisis. Because of the novelty of the virus, there are currently no SARS-CoV-2–specific treatments or vaccines available. Therefore, rapid development of effective vaccines against SARS-CoV-2 are urgently needed. Here, we developed a pilot-scale production of PiCoVacc, a purified inactivated SARS-CoV-2 virus vaccine candidate, which induced SARS-CoV-2–specific neutralizing antibodies in mice, rats, and nonhuman primates. These antibodies neutralized 10 representative SARS-CoV-2 strains, suggesting a possible candidate SARS-CoV-2 vaccine, we isolated SARS-CoV-2 strains from bronchoalveolar lavage fluid samples of 11 hospitalized patients (including five patients in intensive care), of which five are from China, three from Italy, one from Switzerland, one from the United Kingdom, and one from Spain (table S1). These patients were infected with SARS-CoV-2 during the most recent outbreak. The 11 samples contained SARS-CoV-2 strains that are widely scattered on the phylogenetic tree constructed from all available sequences, representing to some extent circulating SARS-CoV-2 populations (Fig. 1A and fig. S1). We chose strain CN2 to develop a purified inactivated SARS-CoV-2 virus vaccine, PiCoVacc, and another 10 strains, CN1, CN3 to CN5, and OS1 to OS6, as preclinical challenge strains. The CN1 and OS1 strains are closely related to 2019-nCoV-BetaCoV Wuhan/ WIV04/2019 and EPI_ISL_412973, respectively, which have been reported to cause severe clinical symptoms, including respiratory failure requiring mechanical ventilation (9, 10).

To obtain a viral stock adapted for efficient growth in Vero cells for PiCoVacc production, the CN2 strain was first plaque purified and passed once in Vero cells to generate the P1 stock. After this, another four passages were performed to generate the P2 to P5 stocks. Growth kinetics analysis of the P5 stock in Vero cells showed that this stock replicated efficiently and reached a peak titer of 6 to 7 logs10 median tissue culture infectious dose (TCID50)/ml by 3 or 4 days post-infection (dpi) at multiplicities of infection of 0.0001 to 0.01 and temperatures between 33°C and 37°C (Fig. 1B). To evaluate the genetic stability of PiCoVacc, five more passages were performed to obtain the P10 stock, and its whole genome, together with those of the P1, P3, and P5 stocks, was sequenced. Compared with P1, only two amino acid substitutions, Ala→Asp at E residue 32 (E-A32D) and Thr→Ile at nspl0 residue 49 (nspl0-T49I), occurred in the P5 and P10 stocks (table S2), suggesting that the PiCoVacc CN2 strain has excellent genetic stability without the S mutations that might potentially alter the NAb epitopes. To produce pilot-scale PiCoVacc for animal studies, the virus was propagated in a 50-liter culture of Vero cells using the Cell Factory system and inactivated using β-propiolactone (Fig. 1C). The virus was purified using depth filtration and two optimized steps of chromatography, yielding a highly pure preparation of PiCoVacc (Fig. 1D). Additionally, cryo–electron microscopy analysis showed intact, oval-shaped particles with diameters of 90 to 150 nm, which were embellished with crown-like spikes, representing a prefusion state of the virus (Fig. 1E).

To assess the immunogenicity of PiCoVacc, groups of BALB/c mice (n = 10) were injected at days 0 and 7 with various doses of PiCoVacc mixed with alum adjuvant (0, 1.5, 3, or 6 µg per dose, with 0 µg in physiological saline as the sham group). No inflammation or other adverse effects were observed. Spike-specific, receptor binding domain (RBD)–specific, and N-specific antibody responses were evaluated by enzyme-linked immunosorbent assays (ELISAs) at weeks 1 to 6 after the initial immunization (fig. S2).

**Corresponding author.** Email: qinchuan@pumc.edu.cn (C.Q.); yjzhang@icdc,zj.cn (Y.Z.); yinwd@sinovac.com (W.Y.); chuangqin@icdc.cn (J.L.)

**Division of Respiratory Virus Vaccines, National Institute for Food and Drug Control, Beijing, China.**

**CAS Key Laboratory of Infection and Immunity, National Laboratory of Macromolecules, Institute of Biophysics, Chinese Academy of Sciences, Beijing, China.**

**National Institute for Communicable Disease Control and Prevention, Chinese Center for Disease Control and Prevention, Changping, Beijing, China.**

**Institute of Microbiology and Epidemiology, Academy of Military Medical Sciences, Beijing, China.**

**These authors contributed equally to this work.**
Fig. 1. Characterization of the SARS-CoV-2 vaccine candidate PiCoVacc. (A) SARS-CoV-2 maximum likelihood phylogenetic tree. The SARS-CoV-2 isolates used in this study are depicted with black lines and labeled. Viral strains were isolated in infected patients who traveled from the continents indicated. (B) Growth kinetics of PiCoVacc (CN2) P5 stock in Vero cells. (C) Flowchart of PiCoVacc preparation. (D) Protein composition and purity evaluation of PiCoVacc by NuPAGE 4 to 12% Bis-Tris gel. (E) Representative electron micrograph of PiCoVacc. White scale bar 100 nm.

Fig. 2. PiCoVacc immunization elicits an NAb response against 10 representative SARS-CoV-2 isolates. BALB/c mice and Wistar rats were immunized with various doses of PiCoVacc or control (adjuvant only) (n = 10). Sera from recovered COVID19 patients (RECOV) and noninfected (NI) individuals were used as positive and negative controls, respectively. The antibody responses were analyzed in mice (A), humans (B), and rats (C). Top: SARS-CoV-2–specific IgG responses as measured by ELISA. Bottom: NAb titer as determined by microneutralization assay. The spectrum of neutralizing activities elicited by PiCoVacc was investigated in mice (D) and rats (E). Neutralization assays against the other nine isolated SARS-CoV-2 strains were performed using mouse and rat serum collected 3 weeks after vaccination. Data points represent mean ± SEM of individual animals and humans from five to 10 independent experiments. Error bars indicate SEM. Dotted lines indicate the limit of detection. Horizontal lines indicate the geometric mean titer of median effective concentration (EC₅₀) for each group.
respectively, at week 3 (before virus challenge) in both vaccinated groups; their titers were similar to those of sera from the recovered COVID-19 patients (Fig. 3, A and B). Unexpectedly, NAb titers (61) in the medium-dose group were ~20% greater than those (50) observed in the high-dose group at week 3, possibly because of individual differences in the ability of one animal in the medium-dose group in eliciting an ~10-fold higher titer compared with the other three animals (Fig. 3B). Excluding this exception, the NAb titer in the medium-dose group decreased to 34, ~40% lower than that in the high-dose group. Subsequently, we conducted a challenge study by a direct inoculation of 10^6 TCID_{50} of SARS-CoV-2 CN1 into the trachea of macaques at 369 dpi, along with severe interstitial pneumonia (Fig. 3, C to F). By contrast, all vaccinated macaques were largely protected against SARS-CoV-2 infection, with very mild pneumonia (Fig. 3, C to F). By contrast, all vaccinated macaques were largely protected against SARS-CoV-2 infection, with very mild

Fig. 3. Immunogenicity and protective efficacy of PiCoVacc in nonhuman primates.

Macaques were immunized three times intramuscularly with various doses of PiCoVacc or adjuvant only (sham) or placebo (n = 4). SARS-CoV-2–specific IgG response (A) and NAb titers (B) were measured. Data points represent mean ± SEM of individual macaques from four independent experiments. Error bars indicate SEM. Dotted lines indicate the limit of detection. Horizontal lines indicate the geometric mean titer of EC_{50} for each group. (C to F) The protective efficacy of PiCoVacc against SARS-CoV-2 challenge at week 3 after immunization was evaluated in macaques. Viral loads of throat (C) and anal (D) swab specimens collected from the inoculated macaques at 3, 5, and 7 dpi were monitored. Viral loads in various lobes of lung tissue from all the inoculated macaques at 7 dpi were measured (E). RNA was extracted and viral load was determined by quantitative reverse transcription polymerase chain reaction. All data are presented as means ± SEM from four independent experiments. Error bars indicate SEM. Asterisks indicate significance: *P < 0.05 and **P < 0.01. (F) Histopathological examinations in lungs from all the inoculated macaques at 7 dpi. Lung tissue was collected and stained with hematoxylin and eosin.

and focal histopathological changes in a few lobes of lung, probably caused by a direct inoculation of 10^6 TCID_{50} of virus into the lung through the intratracheal route that needed a longer time (>1 week) to recover completely (Fig. 3F). Viral loads decreased significantly in all vaccinated macaques but increased slightly in control animals at 3 to 7 dpi (Fig. 3, C to E). All four macaques that received the high dose had no detectable viral loads in pharynx, crissum, or lung at 7 dpi. In the medium-dose group, we indeed partially detected the viral blip from pharyngeal (3/4), anal (2/4), and pulmonary (1/4) specimens at 7 dpi, whereas viral loads presented an ~95% reduction compared with the sham groups (Fig. 3, C to E). The NAb titer in vaccinated groups decreased by ~30% by 3 dpi to neutralize viruses, then rapidly increased from 5 to 7 dpi to maintain neutralization efficacy. Compared with the high-dose group (titer of ~145), the higher NAb titers observed in the medium-dose group at 7 dpi (titer of ~400 for four macaques) might have resulted from relatively low levels of viral replication, suggesting that a longer time was required for complete viral clearance. No antibody-dependent enhancement (ADE) of infection was observed for the vaccinated macaques despite the observation that a relatively low NAb titer existed within the medium-dose group before infection, offering partial protection. The possibility of manifestation of ADE after antibody titers wane could not be ruled out in this study. Further studies involving observation of challenged animals at longer periods of time after vaccination are warranted to address this.
Evaluate lung evaluations in lungs from four groups of macaques at day 29. Lung tissue was collected and stained to show mean ± SD from four independent experiments. Error bars indicate SD. (A) Percentage of lymphocytes, including CD3+, CD4+, and CD8+, were monitored by intramuscular injection with low (1.5 μg per dose) or high-dose (6 μg per dose) PiCoVacc or adjuvant only (sham) or placebo. (A and B) Hematological analysis in all four groups of macaques (n = 4). (A) Percentage of lymphocytes, including CD3+, CD4+, and CD8+, were monitored at days −1 (1 day before vaccination), 18 (3 days after the second vaccination), and 29 (7 days after the third vaccination). (B) Key cytokines containing TNF-α, IFN-γ, and IL-2 were examined at days −1, 1 (the day of the first vaccination), and 4, 18, and 29 after vaccination. Data points show mean ± SD from four independent experiments. Error bars indicate SD. (C) Histopathological evaluations in lungs from four groups of macaques at day 29. Lung tissue was collected and stained with hematoxylin and eosin.

**Fig. 4. Safety evaluation of PiCoVacc in nonhuman primates.** Macaques were immunized three times at days 0, 7, and 14 intramuscularly with low-dose (1.5 μg per dose) or high-dose (6 μg per dose) PiCoVacc or adjuvant only (sham) or placebo. (A and B) Hematological analysis in all four groups of macaques (n = 4). (A) Percentage of lymphocytes, including CD3+, CD4+, and CD8+, were monitored at days −1 (1 day before vaccination), 18 (3 days after the second vaccination), and 29 (7 days after the third vaccination). (B) Key cytokines containing TNF-α, IFN-γ, and IL-2 were examined at days −1, 1 (the day of the first vaccination), and 4, 18, and 29 after vaccination. Data points show mean ± SD from four independent experiments. Error bars indicate SD. (C) Histopathological evaluations in lungs from four groups of macaques at day 29. Lung tissue was collected and stained with hematoxylin and eosin.

世界范围内的COVID-19大流行和随之而来的死亡率持续上升，全球范围内的疫苗研发和接种仍然是当务之急。
Vaccine candidate tested in monkeys

Global spread of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has led to an urgent race to develop a vaccine. Gao et al. report preclinical results of an early vaccine candidate called PiCoVacc, which protected rhesus macaque monkeys against SARS-CoV-2 infection when analyzed in short-term studies. The researchers obtained multiple SARS-CoV-2 strains from 11 hospitalized patients across the world and then chemically inactivated the harmful properties of the virus. Animals were immunized with one of two vaccine doses and then inoculated with SARS-CoV-2. Those that received the lowest dose showed signs of controlling the infection, and those receiving the highest dose appeared more protected and did not have detectable viral loads in the pharynx or lungs at 7 days after infection. The next steps will be testing for safety and efficacy in humans.

Science, this issue p. 77