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SARS-CoV-2 Delta Inactivated Vaccine: Safety Assessment in Sprague Dawley Rats and Cynomolgus Monkeys

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ABSTRACT

Developing a reliable and safe SARS-CoV-2 vaccine is essential to halt the COVID-19 pandemic. Preclinical studies are designed to evaluate vaccine safety. Testing vaccine toxicity in animals helps predict safety in humans, thereby reducing risks during human use and providing a foundation for clinical trial design. This study aimed to (1) provide a reference for clinical research by assessing the toxicity of a SARS-CoV-2 inactivated vaccine (Vero cell line, Delta strain B.1.617.2) following six weeks of repeated intramuscular injections, and (2) evaluate the acute toxicity of the vaccine in Sprague-Dawley rats over 14 days after a single intramuscular injection. Repeated intramuscular injections in cynomolgus monkeys revealed elevated body temperature, eosinophils, fibrillation, and cytokine IL-6 levels after the first and/or last injection, with no evident systemic damage; five doses per monkey were deemed safe. Injection site irritation was observed at one and five doses per monkey, with recovery noted after four weeks. High levels of specific IgG antibodies against the S1 protein were detected in all animals. These findings suggest the vaccine is safe and support progression to human phase I clinical trials. Additionally, this study provides a protocol for developing a preclinical evaluation system for next-generation SARS-CoV-2 vaccines.

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Introduction

Coronaviruses are common in nature and can cause the common cold and other illnesses[1-4]. Continuous mutations of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) lead to the continued occurrence of breakthrough infections with different SARS-CoV-2 variants [5]. SARS-CoV-2, the pathogen of COVID-19, is a single-stranded RNA coronavirus strain in humans. In 2020, a global outbreak of SARS-CoV-2 spread across the world[6]. Since the start of the pandemic, the spread of the COVID-19 has had an enormous impact globally[7].

Vaccination can effectively prevent the spread of COVID-19 and plays an important role in the prevention and control of the pandemic[8-10]. Nowadays, a variety of vaccines against SARS-CoV-2 (inactivated vaccines, recombinant protein vaccines, adenovirus vector vaccines, DNA vaccines, and RNA vaccines) have been developed[11, 12]. However, SARS-CoV-2 mutations might diminish vaccine-induced protective immune responses[13]. CoronaVac or BBIBP-CorV showed only 59% protection against the SARS-CoV-2 variant Delta (B.1.617)[14, 15]. One dose and two doses he AZD1222 are estimated to be 30% and 67% effectiveness for one and two doses of the Delta variant, respectively[16]. This highlights the urgent need to accelerate the development of a vaccine against the Delta variant.

Previous research has shown that people who get only a single dose of the vaccine are susceptible to Delta, while a second dose provides better protection[16-18]. Similarly, Omicron variant (B.1.1.529) has established itself as the primary variant over the course of the past few months, demonstrating exceptional communicability and contagiousness relative to other variant of concerns (VOCs)[19]. Nevertheless, when analyzing the mutation sites on the S protein and the S-ACE2 complex structure, some studies found that although there were a large number of mutations in the S protein on both the omicron mutant and the delta mutant, the interaction between the S protein and H-ACE2 Patterns remain conservative [20].

Furthermore, vaccines based on the original SARS-CoV-2 Spike could still significantly improve immunity against Omicron[21-23]. Therefore, we have developed an inactivated vaccine for Delta variant and induced it to produce high-level neutralized antibodies in the animal to prevent SARS-CoV-2. Correspondingly, it is also hoped to develop valuable information for scientists to develop vaccines against Omicron.

Healthy people are the target demographic for the SARS-CoV-2 inactivated vaccine; therefore, safety studies at the preclinical stage are very important[24]. Testing the vaccine's toxicity in animals can help to anticipate the vaccine's safety for human use, which can considerably lower the risk during

human use and form the basis for the design of clinical trials. The present study aimed to perform a thorough preclinical safety evaluation of the SARS-CoV-2 inactivated vaccine, including single-dose and repeated-dose toxicity experiments in rats and cynomolgus monkeys.

Materials and methods

1. Vaccine sources

All of the SARS-CoV-2 strains used in this study were collected in 2021 from patients diagnosed with COVID-19 who were hospitalized at Zhoushan Hospital in Zhejiang Province, China, and saved in the Zhejiang Provincial Center for Disease Control. This study was approved by the Ethical Review Committee of Zhejiang Provincial Center for Disease Control and Prevention, China (2021-002-01).

The Zhejiang Provincial Center for Disease Control and the Ningbo Rongan Biopharmaceutical Co., Ltd. have collaborated to create an inactivated vaccine against SARS-CoV-2(2021-002-01). In brief, Vero cells were infected with a virus strain called new coronavirus inactivated vaccine (Vero cell, Delta strain, GISAID number: EPI_ISL_1911196) and then the vaccine was manufactured in a Biosafety level (BSL)-compliant setting. The vaccine specification is 0.5mL/dose and contains aluminum hydroxide adjuvant (0.5mg/dose, calculated in terms of aluminum content), NaCl, Na2HPO4, NaH2PO4 ·H2O and the SARS-CoV-2 inactivated antigen.

2. Animal study

The Sprague-Dawley (SD) rats were specific pathogen free at the beginning of administration (D1), were aged about 6–7 weeks, and weighed 189–204 g (male) and 184–198 g (female) at D1. The animals are provided by Beijing Weitong Lihua Experimental Animal Technology Co., Ltd. (Beijing, China), production license number SCXK (Beijing) 2016-0011, Institutional Animal Ethics Committee (IACUC) approval number: ACU21-2368.

During the experiment, the rats were kept in polycarbonate boxes with no more than five animals/sex in each cage. The experiments were carried out following the guidelines for the use and management of laboratory animals, Edition 8 [25] and the U.S. Department of Agriculture Animal Welfare Act [26].

Cynomolgus monkeys: The specific pathogen free cynomolgus monkey were 3–5 years old and weighed 2.22–3.28 kg (female) and 2.99–5.13 kg (male) when purchased. The monkeys were provided by Guangxi Frontiers Biotechnology Co., Ltd. (Nanning, China), production license No. SCXK (Gui) 2021-0001, laboratory animal quality certificate license No.0009403, IACUC approval number: ACU21-2324.

During the test period, the cynomolgus monkeys were fed with fruit (at about 50 g/day) and qualified monkey feed every day

at about 200 g/day. The use of experimental animals in this study was approved by the Beijing Municipal Commission of Science and Technology, use license NO. SYXK (Jing) 2019-0006.

The combination of the studies in accordance with 3R principles allowed a reduction of experimental animals. The study is in com- compliance with the ARRIVE guidelines.

3. Toxicity test design

The Provantis 9.4.3.0 system (Instem, Stone, UK) was used to randomly sort the animals into several categories, based on their sex. Table 1 displays the details of the classifications used.

Table 1 Grouping and administration

Group	Dosage[a] (Dose/rat or monkey)	Capacity (mL/rat or monkey)	Number of animals (Rat or monkey/sex)	Animal number	
				Male	Female
Group 1 (Negative control group)	0	2.5	3+2[b]	2120631~2120635	2120636~2120640
Group 2 (Low dose of test sample)	1	0.5	3+2[b]	2120641~2120645	2120646~2120650
Group 3 (High dose of test sample)	5	2.5	3+2[b]	2120651~2120655	2120656~2120660
Group 4 (Negative control group)	0	-	5	2120781~2120785	2120786~2120790
Group 5 (Test sample)	4	2[c]	5	2120791~2120795	2120796~2120800

Note: **a:** In this experiment, the dose unit is "dose per rat or monkey", and each dose is equivalent to the dose per person in clinic. when given to the test group, the solution is extracted according to the volume of each bottle of 0.5mL, and the rest of the bottle is disposed of according to medical waste after 0.5mL is extracted. when the capacity of one bottle is less than 0.5mL, it is shaken from another bottle and then supplemented and extracted to 0.5mL for drug administration. **b:** 3 monkeys/sex/group were dissected at the end of 4-week administration period (D46), and the latter 2 monkeys/sex/group were dissected at the end of 4-week recovery period (D71). c: The concentration of the test samples in this group is 20 μg/mL.

3.1 Single-dose toxicity study in rats

Twenty SD rats (10/sex) were randomly assigned into two groups (5/sex/group): the negative control group and the test group. The animals in the negative control groups were given sodium chloride injection (2 mL/rat) and adjuvant (2 mL/rat), and the animals in the test group were given four doses of the novel coronavirus inactivated vaccine (Vero cell, Delta strain) by intramuscular injection. The administration sites of all animals were the gastrocnemius and quadriceps muscles of both hindlimbs. The medicine volume is 2 mL/rat (20 μg/mL).

All the animals mentioned above were injected with 0.25 mL at each point. During the trial, the death / near death, clinical symptoms, body weight, and food intake of the animals were observed and recorded. All animals were euthanized on D15 for gross anatomical observation, fixation of abnormal tissue, and histopathological examination.

SD rats were euthanized by exsanguination via the femoral artery after anesthesia with CO_2 inhalation, in group order.

3.2 Cynomolgus monkey repeat-dose toxicity study

A total of 30 cynomolgus monkeys were used in the experiment. According to the body weight of the animals (measured 4 days before administration), the animals were randomly divided into groups by sex using the Provantis 9.4.3.0 system. Five male and five female monkeys were used to create a control group, a low-dose group, and a high-dose group.

The administration route of each group was single-point or multi-point intramuscular injection of quadriceps femoris in the right hindlimb, and the injection capacity did not exceed 0.5 mL at each point. The animals in the negative control groups were given sodium chloride injection (2.5 mL/monkey) and adjuvant (2.5mL/monkey), the low and high dose groups were given novel coronavirus inactivated vaccine (Vero cells, Delta strain) at one dose (0.5 mL/monkey) and five doses (2.5 mL/monkey), respectively, and were administered at D1, D15, D29, and D43 for a total of four times. Cynomolgus monkeys were euthanized with ketamine (10 mg/kg, 0.2 mL/kg, 50 mg/mL) (Sigma-Aldrich) administered via intramuscular injection in the left hind limb, followed by intramuscular injection of haloperidol hydrochloride injection (1 mg/kg, 20 mg/mL) in the left hind limb, three days after the last dosing (D46) and at the end of the recovery period four weeks later (D71). After the animals reached unconsciousness, exsanguination via the femoral artery was performed to euthanize the animals.

4. Animal in vivo detection index

All animals were checked twice a day during the trial (once in the morning and once in the afternoon). We have carried out local observation t before, and the next day after, each administration, and local

observation was carried out at the same time during the detailed clinical observation. The day before the first injection, and once a week thereafter, the cynomolgus monkeys were released from their cages for intensive clinical observation. SD rats were taken out for detailed clinical observation 1 day before administration, and at 7 and 14 days after administration.

4.1 Body weight and food intake

During the experiment, all rats were fed with feed at least once a week after administration, and the body weight was measured at 4, 7, 11, and 14 days. All cynomolgus monkeys were weighed once a week, the day before the first administration, and after the start of administration. Animals are also weighed when euthanized.

The weight change was calculated as follows:

Weight gain percentage(%)=100×(Current weight-Weight before administration)/ Weight before administration.

All rats were fed at least once a week after administration, and the amount of added feed was recorded. The uneaten feed was measured twice a week (at D4, D7, D11, D14, respectively, the number of days is equal to the second measurement date minus the first measurement date) and the average food intake (added/surplus) was calculated and expressed in the form of "g/mouse/day". The daily intake of each rat was calculated as follows:

Food intake(%)=100×(Adding amount-Remaining amount)/(Number of animals ×days)

4.2 Temperature

The body temperature of all cynomolgus monkeys was measured on the day before the first administration, 4–6 hours after the first administration, the day after the first administration, 4–6 hours after the end of the 43-day administration, the day after the last administration (D44), and the day before the end of the recovery period (D70).

4.3 Electrocardiogram and blood pressure monitoring

All cynomolgus monkeys were measured using Shanghai photoelectric ECG-6951E electrocardiograph (Shanghai, China) before the first administration (D-2), 4–6 hours after D1 administration, 4–6 hours after the first administration (D2), 4–6 hours after D43 administration, 4–6 hours after the last administration (D44), and 1 day before the end of the recovery period (D70). Heart rate, electrocardiogram (ECG) waveform, P-R interval, QRS duration and Q-T interval. Calculate QTcB according to Bazett formula [27].

All cynomolgus monkeys were treated before the first drug (D-2), 4-6 hours after the end of D1 administration, the next day after the first drug (D2), 4-6 hours after the end of D43 administration, and the next day after the last drug (D44) and 1 day before the end of the recovery period (D70) using the BP-2010E smart non-invasive blood pressure monitor (Beijing Ruanlong

Biological Technology Co., Ltd., BP-2010E) to measure and record the following parameters: mean arterial pressure (MBP, mmHg), systolic and diastolic blood pressure (SBP and DBP, mmHg).

4.4 Eye examination and urine analysis

All cynomolgus monkeys were examined by ophthalmologist before grouping, the day after the last vaccine administration (D44), and the day before the end of convalescent period (D70). The examination included the face, conjunctiva, cornea, iris, pupil, lens, sclera, vitreous, retina, and fundus. A pupil dilator was used during the eye examination.

The fresh urine of cynomolgus monkeys was collected one day before the first administration (D-1), 2 days after the last administration (D45), and one day before euthanasia (D70). Use Cobas6500 urine test analyzer (Roche, Cobas6500) for detection (Roche, Basel, Switzerland).

4.5 Blood cell count, coagulation function, and blood biochemical analysis

Blood samples from the subcutaneous vein of the left hindlimb of the cynomolgus monkeys were collected before the first administration (D-2), 3 days after the first administration (D4), 3 days after the last administration (D46), and at the end of the recovery period (D71). Blood cell count, blood coagulation, and blood biochemical indexes were detected. The monkeys were fasted overnight before taking blood. Three blood samples (1, 2, and 2 mL) were collected and packed in sampling tubes containing EDTA anticoagulant (for the blood cell count), sodium citrate (for the blood coagulation test) and no anticoagulant (for the blood biochemical determination). An automatic blood cell analyzer (Siemens, ADVIA 2120i), a SYSMEX blood coagulation analyzer (SYSMEX, CS-1300), and a TBA-120FR automatic biochemical analyzer (TOSHIBA, TBA-120FR) were used to detect blood cell count, blood coagulation function, and blood biochemical indexes, respectively.

5. Detection of immunological indexes

5.1 T lymphocyte subsets

The left hind limb subcutaneous vein was pricked before (D-2), after (D-4), and three days after (D-46) administration in groups 1 to 3, and again after the recovery period (D-71) (using the same sample as the blood cell count). A Beckman DxFLEX flow cytometry (Beckman Coulter, Indianapolis, IN, USA) was used to determine the proportion of cluster of differentiation (CD)3+, CD3+CD4+, and CD3+CD8+ T cells in the peripheral blood and to compute the CD3+CD4+/CD3+CD8+ ratio.

5.2 Cytokines and C-reactive protein

In groups 1–3, the blood samples of left hindlimb subcutaneous vein were collected before the first administration (D-2), 4–6 hours after D1 administration, 4–6 hours after the first administration (D2), 4–6 hours after D43 administration, the day after the last administration (D44), and the end of recovery period (D71). Blood samples were

taken at the same testing time points. Blood samples were collected according to the order of one rat in each group. The content of serum C-reactive protein was determined using the TBA-120FR automatic biochemical analyzer (using immune turbidimetry). The remaining serum was stored below -20 ℃ and used for cytokine assays.

After all blood samples were collected, tumor necrosis factor-alpha (TNF-α), interferon-gamma (IFN-γ), interleukin (IL)-2, IL-4, IL-5, and IL-6 were detected by the CBA kit (Becton,Dickinson and Company, Cat: 557800) and Beckman DxFLEX flow cytometer.

5.3 Complement

In the crab monkey group, the blood samples of left hindlimb subcutaneous vein (1 mL) were collected before the first administration (D-2), the day after the first administration (D2), the day after the last administration (D44), and the end of the recovery period (D71). Collect 1 mL of subcutaneous venous blood sample from the left hindlimb, and use blood biochemical or C-reactive protein samples at the same detection time point.

Determination of serum complement C3 and C4 was carried out using the TBA-120FR automatic biochemical analyzer (using the immune turbidimetric method)

5.4 Serum-specific IgG antibodies

A coated antigen consisting of a Recombinant SARS-Cov-2 Spike S1 Protein (His Tag) was diluted with carbonate buffer and added to the wells of a 96-well plate, which was then incubated overnight. After washing the plate, the wells were sealed and incubated with shaking for 1 hour.

Serum samples were diluted with sample diluent and added to the wells at various dilutions. After incubation, a rabbit anti-monkey IgG labeled with horseradish peroxidase was added, followed by a chromogenic solution and Terminator.

The optic density (OD) value was measured at 450 nm, and if the OD value of the animal serum in the treatment group was ≥ the cut-off value, it was judged to be antibody positive, otherwise, it was negative. The serum dilution multiples of the other groups at the D14 time point were 1:100, 1:200, 1:400, 1:1600, 1:3200, 1:12800, 1:51200 and 1:102400. The dilution multiples of serum at D28, D42, D57, and D71 were 1:1600, 1:3200, 1:12800, 1:51200, 1:102400, 1:204800, 1:409600, and 1:819200, and were added at 100 μl / well.

6. Gross and histopathological examination

According to the American Veterinary Medical Association (AVMA) Guidelines for the euthanasia of animals: 2020 edition[28], 3 days after the last vaccine administration (D46) and 4 weeks after the end of the recovery period (D71), the cynomolgus monkeys in groups 1–3 were injected with ketamine (10 mg/kg) (0.2 mL/kg and 50 mg/mL) through the left hindlimb muscle, and then simazine hydrochloride (1

mg/kg/mL) was injected into the left hindlimb muscle. After the animals entered the unconscious state, they were euthanized by bloodletting through the femoral artery. The same operation was performed on the rats at D15.

Systematic autopsies were performed on all animals that were euthanized. The observations included but were not limited to, whether there are any abnormalities in the animal's body surface, orifices, head, chest, abdomen, or pelvis. The gross anatomy and injury results were recorded. Damaged or abnormal tissues or organs were fixed in 10% neutral formalin solution.

Sternal bone marrow smears were prepared from cynomolgus monkeys in groups 1–3s (no obvious change of bone marrow toxicity was found in blood cell count; therefore, they were not examined under the microscope). The organ weight/body weight ratio (organ-body ratio) and organ weight/brain weight ratio (organ-brain ratio) was calculated by weighing the fasting body weight and organ weight of all euthanized monkeys.

Routine histological treatments, including paraffin embedding, sectioning, lamination, hematoxylin and eosin (HE) staining, and histopathological inspection, were applied to the gross anatomy of the euthanized animals in each group to identify organ and tissue aberrations that might be connected to the vaccine delivery. Microscopic damage is rated on a five-grade system (minor, mild, moderate, severe)

7. Statistical analysis

GraphPad Prism software V6 and Biorender were used for statistical analyses and graphic drawing. All statistical testing was performed using a two-tailed test with a significance level of 5% (P = 0.05). Body weight, body weight gain, temperature, ECG parameters, blood pressure, clinico--pathological indices (blood cell count, blood coagulation function, blood biochemical indices), T lymphocyte subsets, complement, C-reactive protein, cytokines, organ weight, organ-body ratio, and organ-brain ratio averages and standard deviations were all calculated in the Provantis system.

Male and female animals were counted independently, and the results were compared with those of the control group that had not been subjected to the experiment. The following methods were used to analyze the data: To determine if the variance was normally distributed, we first used Levene's Test; if the variance was normally distributed, we ran a one-way analysis of variance (ANOVA) test; and if the difference between the two groups was statistically significant, we ran Dunnett's multiple comparisons (parameter technique).

If the variance was uneven, Log transformation (Ln transformation) was performed on the data, followed by Levene's Test. A one-way ANOVA test was performed if the data were homogenous after Log

transformation, and Dunnett's multiple comparisons (parameter method) was performed if the ANOVA difference was significant. If the variance of the data was still uneven after Log conversion, the Kruskal–Wallis nonparametric test was carried out on the original data. Two-way comparisons were made using the Mann–Whitney U test if the Kruskal–Wallis nonparametric test yielded a statistically significant result. When there was a negative value in the data, there was no "Log conversion", the data is only "no conversion" or "Rank conversion". When there was a 0 in the data, the "0" value was taken as the smallest positive value in the data, and then Log conversion was carried out. If the number of samples (n) was less than 3, no statistical analysis was carried out.

8. Ethics

The use of experimental animals in this study was approved by the Beijing Municipal Commission of Science and Technology (Approval Number: ACU21-2368).

Results

1.Clinical observation and local observation of drug administration

Each group showed no signs of mortality or near death during the trial, and there was no visible evidence of erythema, hyperemia, edema, ulcer, or induration at the site of injection. In addition to the general clinical observation twice a day, the allergic reaction symptoms of animals were also closely observed in the clinical observation after each administration. No allergic reactions such as skin pruritus, erythema, papules, eyelid swelling, or abnormal breathing were found in all groups.

In the high dose group (5 doses/monkey), a moderate amount of soft stool or a small amount of thin yellow stool could be seen at D-2 to D-1 and D35 to D46 of one male cynomolgus monkey (2120653), a small amount of yellow dilute stool at D36 to D42 of one female cynomolgus monkey (2120658) was observed, and a small amount of yellow dilute stool at D28-D30 and D36 to D37 of one cynomolgus monkey (2120660) was observed.

No obvious abnormal reactions were found in the clinical observation of the other monkeys. Although the symptoms of loose stool in the above animals were only seen in the high-dose group, recovery was observed in two female animals and before the first administration in the male animals, which was not related to vaccine administration.

2. Changes in body weight and food intake

SD rats: The statistical data of the weight changes of the rats in the two groups are shown in Figure 1A. During the experiment, there was no significant difference in the body weight of the test group and the control group animals. ($P > 0.05$). Individual data on average food consumption are shown in Figure 1B. During the experiment, compared with the same-sex negative control group,

there was no significant abnormal change in the food intake of the animals in the test group ($P > 0.05$).

Cynomolgus monkeys: The statistical data on the weight changes of the cynomolgus monkeys are shown in Figure 2A. During the experiment, the body weight of animals in each group fluctuated over a small range, and during the experiment, compared with the negative control group of the same sex in the same period, there were no significant changes in body weight and body weight growth in low (1 dose) and high dose groups ($P > 0.05$). That is, there is no effect of vaccine administration on the body weight of the animals.

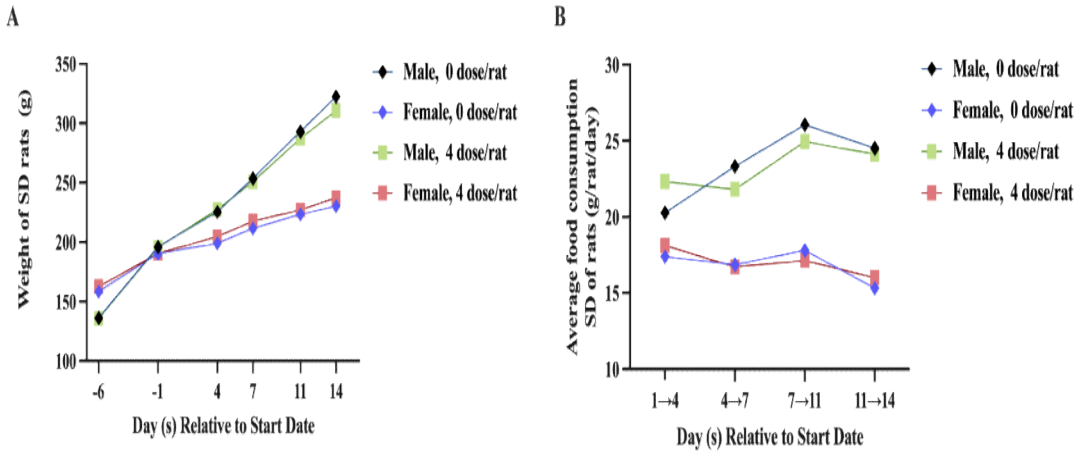

Figure1: Changes in body weight and food intake of SD rats.

During the experiment, there was no significant difference in the body weight and food intake of the test group and the control group animals ($P > 0.05$). (A) -6: the 6 days before the injection vaccine relative to start date (on the day of injection vaccine). Male, 0 dose/rat: the negative control group, each male rat don't give vaccine injection. Male 4 dose/rat: test sample group, each male rat is given 4 doses vaccines injection. (B) 1 → 4 means the number of days, that is, the second measuring date minus the first measurement date. SD rats are determined by the amount of surplus material in D4, D7, D11, D14 and calculated the average feeding amount (adding amount/remaining amount), and expressed in the form of "g/rat/day".

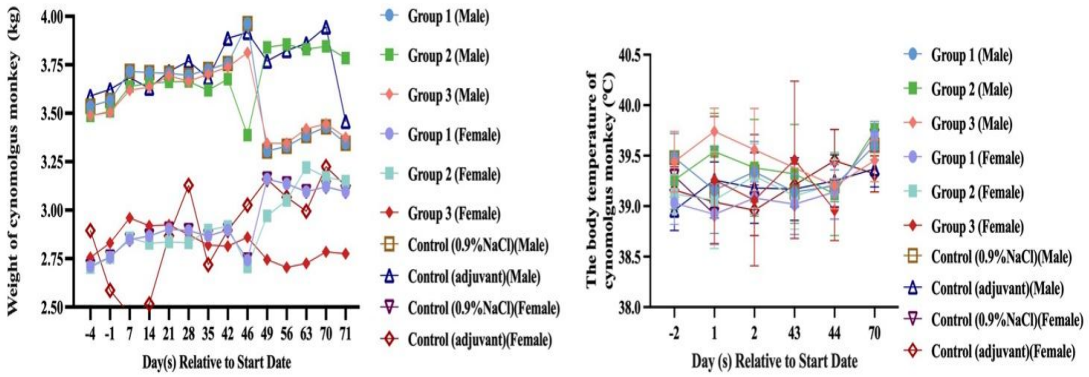

Figure2: Changes in body weight and body temperature of cynomolgus monkeys.

-4: the 4 days before the injection vaccine relative to start date (on the day of injection vaccine).Group 1 (Male): the negative control group, each cynomolgus monkeys don’t give vaccine injection; Group 2 (Male): the low dose of test sample group, each cynomolgus monkey is given 1 dose vaccine injection; Group 3 (Male): the high dose of test sample group, each cynomolgus monkey is given 5 dose vaccine injection. During the experiment, compared with the same sex negative control group, there were no significant changes in blood pressure indexes ($P > 0.05$).

3. Body temperature changes

Body temperature statistics of cynomolgus monkeys are shown in Figure 2B. During the experiment, the body temperature of each group fluctuated over a small range, and compared with the same-sex negative control group in the same period, the main changes ($P \leq 0.05$) of body temperature were as follows: At 4–6 hours after the last vaccine administration (D43), the body temperature of the male animals in the high dose group increased, and the average value compared With the control group was 39.80 vs. 39.14 ℃.

Individual data showed that the body temperature of individual animals was ≥ 40 ℃ in the low and high-dose groups at 4–6 hours after the first and the second day after the first dose (D1, D2, D43). A recovery trend was observed on the next day, and the increase in body temperature of the above animals might be related to the testing sample.

4. Changes in blood pressure and electro--cardiographic parameters

The statistical data of blood pressure parameters of the cynomolgus monkeys are shown in Figure 3. During the experiment, compared with the same-sex negative control group, there were no significant changes ($P > 0.05$) in blood pressure indexes, including systolic blood pressure (SBP), diastolic

blood pressure (DBP), and mean arterial pressure (MBP) in the low and high dose groups. Thus, there was no effect of drug administration on animal blood pressure.

The statistical data of ECG parameters of the cynomolgus monkeys are shown in Table S1. During the experiment, the ECG of each group was in sinus rhythm, and the ECG waveform was normal. Compared with the same sex negative control group in the same period, only on the second day after the first treatment (D2) was the QRS duration of the female animals in the low dose group significantly shorter than that in the control group ($P \leq 0.05$), the average value was 0.044 vs. 0.050s. The other ECG parameters, including heart rate, PR interval, QRS duration, and QTcB showed no significant change ($P > 0.05$).

There was no dose-response correlation and time-response correlation, or consistency between males and females; therefore, we considered that the observed change had no toxic significance.

5. Ophthalmic examination

No abnormalities were found in the ophthalmological examinations of the cynomolgus monkeys in any of the groups before grouping (D-8/D-6), the day after the last vaccine administration (D44), or at the conclusion of the recovery period (D70).

6. Urine analysis

Individual data of the urine examinations are shown in Table S2. Compared with the data of the animals in the same sex negative control group before treatment (D-1) and the same sex in the same period, there were no obvious abnormal changes related to vaccine

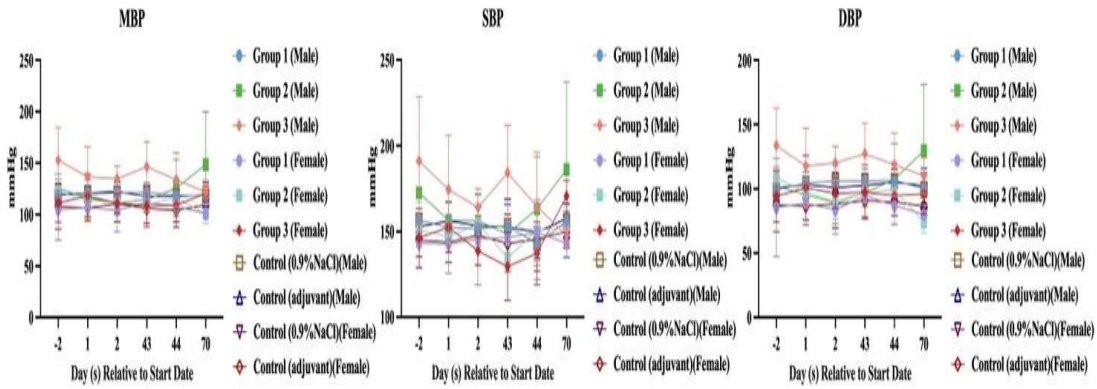

Figure 3. The statistical data of blood pressure parameters of cynomolgus monkeys

-2, the 2 days before the injection vaccine relative to start date (on the day of injection vaccine);1, the first injection time;2, the 2 days after the injection vaccine relative to start date.

MBP: mean arterial pressure; SBP: systolic blood pressure; DBP: diastolic blood pressure.

administration in the low and high dose groups.

7.Clinicopathological examination

7.1 Blood cell count and blood coagulation test

The statistics of the blood cell count of the cynomolgus monkeys are shown in Table S3. During the trial, the monocyte (Mono) of male animals and eosinophils (Eos) of female animals in the high dose group increased considerably 3 days after the previous dose (D46) ($P \leq 0.05$). The values of the Mono (%) and Eos (109/L) comparison between the test group and the control group are 6.28% vs. 4.22 % and 0.640 × 109/L vs. 0.248 × 109/L. The Mono (%) of the female animals in the low and high dose groups decreased significantly ($P \leq 0.05$). The values of the Mono comparison between low dose group, the high dose group and control group are 4.82 %, 4.84 % and 6.36 %. There was no significant change in other indexes of the blood cell count ($P > 0.05$).

The statistical data of blood coagulation function of the cynomolgus monkeys are shown in Table S4. During the experiment, compared with the same sex negative control group in the same period, the changes of blood coagulation function indexes were as follows: Three days after the first dose (D4), the fibrillation (FIB) of female animals in the low dose group (1 dose/monkey) increased, and the activated partial thromboplastin time (APTT) of the male animals in the high dose group was prolonged, and the average value compared with the control group was 23.48 vs.19.40s. Although there were no significant changes in prothrombin time (PT), APTT, and FIB at the rest of the time pints, FIB in the low and high dose groups showed an increasing trend at the first and third day after the last dose (D4, D46), which was considered to be related to acute phase reaction and/or the immune response induced by the vaccine.

7.2 Blood biochemical indexes

The statistical data of blood biochemical indexes and electrolytes of the cynomolgus monkeys are shown in Tables S5 and S6. During the experiment, the main changes of blood biochemical indexes were as follows: Three days after the first administration (D4), the total protein (TP) of the female animals in the low dose group increased, compared with that in the control group (74.66 vs. 69.90 g/L), and the Aspartate Aminotransferase (AST) and Urea levels of male animals in the high dose group increased. The average values were 53.6 vs. 36.2 U/L and 6.62 vs. 5.50 mmol/L compared with the control group, respectively. The K+ of the female animals in the high dose group decreased, and the average value compared with the control group was 4.252 vs. 4.692 mmol/L. Three days after the last dose (D46), the Glu and Ca of the female animals in the high dose group decreased, and the average values were 3.928 vs. 4.446mmol/L and 2.446

vs.2.654 mmol/L compared with those in the control group, respectively.

8. Related indicators of immune function

The statistical data of T lymphocyte subsets in the cynomolgus monkeys are shown in Figure 4A. T lymphocyte subsets CD3+, CD4+, CD8+ and CD4/CD8 showed no significant changes across the experiment in the low and high dose groups when compared to the same sex negative control group ($P > 0.05$). In other words, the distribution of T lymphocyte subsets in the animals was unaffected by the vaccine.

The statistical data of serum cytokines in the cynomolgus monkeys are shown in Table S7. During the experiment, the detection limits of cytokines IL-2, IL-4, IL-5, IL-6, TNF-α, and IFN-γ were 3.6, 0.9, 0.3, 0.1, 0.4, and 3.3 pg/mL respectively. The changes of serum cytokines were mainly characterized by decreases of TNF-α and IL-4 and increases of IL-2, IL-5 and IL-6.

The statistical data of serum C-reactive protein in the cynomolgus monkeys is shown in Figure 4B. After administration, there was no discernible difference in C-reactive protein levels between the low and high dose groups and the same sex negative control group ($P > 0.05$). In other words, there was no significant effect of vaccine administration on the animals' C-reactive protein levels.

The serum complement statistics of the cynomolgus monkeys are shown in Figure 4C. During the experiment, compared with the same sex negative control group, there were no significant changes in serum complement C3 and C4 in the low and high dose groups ($P > 0.05$). Thus, there was no effect of the vaccine on the animals' serum complement.

The detection of specific IgG antibodies in cynomolgus monkey is shown in Table S8 and Figure 4D. The SARS-CoV-2 inactivated vaccine (Vero cells, Delta strain) was repeatedly injected into cynomolgus monkeys via intramuscular injection, and all animals' antibodies were negative before administration. The specific IgG antibodies against the novel coronavirus S1 protein were produced in all animals after one dose and five doses of each sample.

The antibody level increased significantly after the second dose, and was maintained a high titer up to D71. The high-dose group had a higher antibody titer than the low-dose group. In the pre-drug sample (D-2), all animal antibodies were negative. The antibody titers at D14, D28, D42, D57, and D71 in the low-dose group were 1:400–1:200, 1:12800–1:204800, 1:12800–1:204800, 1:12800–1:204800, and 1:12800–1:51200, respectively. The antibody titers at D14, D28, D42, D57, and D71 in the high dose group were 1:400–1:12800, 1:51200–1:819200, 1:102400–1:819200, 1:102400–1:204800, and 1:51200–1:102400, respectively.

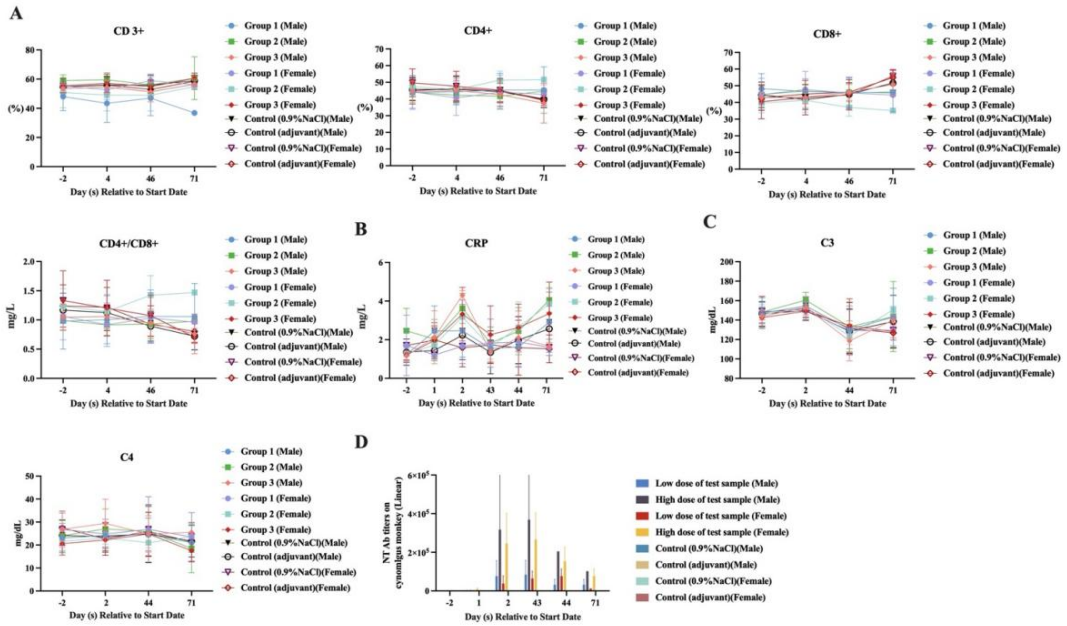

Figure 4. Changes in immune function related indexes in cynomolgus monkeys

(A) The statistical data of Lymphocyte surface antigen of cynomolgus monkeys. CD3+, CD3+ positive T cell; CD4+, CD4+ positive T cell; CD8+, CD8+ positive T cell; CD4+/CD8+, CD4+ positive T cell/CD8+ positive T cell. T lymphocyte subsets CD3+, CD4+, CD8+ and CD4/CD8 showed no significant changes across the experiment in the low and high dose groups when compared to the same sex negative control group ($P > 0.05$). (B) The statistical data of serum C-reactive protein in the cynomolgus monkeys. After administration, there was no discernible difference in C-reactive protein levels between the low and high dose groups and the same sex negative control group ($P > 0.05$). (C) The serum complements statistics of the cynomolgus monkeys. C3, serum complement C3, C4: serum complement C4. Underling the experiment, compared with the same sex negative control group, there were no significant changes in serum complement C3 and C4 in the low and high dose groups ($P > 0.05$). (D) The detection of specific IgG antibodies in cynomolgus monkey. The neutral and antibodies in each group before administration are negative. All animals tested positive after dosing in the low and high dose groups. The antibody titers were 1:400-1:3200, 1:12800-1:204800, 1:12800-1:102400, 1:12800-1:51200 for D14, D28, D42, D57, and D71 in the low dose group. The antibody titers were 1:819200, 1:102400-1:204800, 1:51200-1:102400 for D14, D28, d42, D57, and D71 in the low dose group. D14, 14 days after the first dose; D28, 14 days after the second dose; D42, 14 days after the third dose; D57, 14 days after the fourth dose; D71, recovery period ends in 4 weeks.

9. Pathological examination

9.1 Organ weight/visceral-body ratio/ visceral-brain ratio

The statistical data of organ weight/organ--body ratio/organ-brain ratio of the cynomolgus monkeys is shown in Tables S9-S11. There were no statistically significant differences in organ weight, organ-to-body ratio, or organ-brain ratio between the low and high dose groups and the same-sex negative control group. ($P > 0.05$). Thus, there was no effect of vaccine administration on the organ weight of the animals.

9.2 Gross anatomical observation

All cynomolgus monkeys were euthanized after administration (D46) and recovery (D71), and autopsy revealed no abnormalities.

9.3 Histopathological examination

SD rats: The results of gross dissection and microscopic observation are shown in Figure 5A. The general findings of D15 euthanasia animals and their associated histopathological changes are summarized in Table S12. As can be seen from the table, the results of gross anatomical observation showed that white nodules could be seen in the local administration site of 5 to 10 animals in the test group, and mild to moderate granulomatous inflammation could be seen under the microscope.

Cynomolgus monkeys were euthanized at the end of administration (D46), and histopathological changes related to the samples were observed in the injection area, showing granulomatous inflammation in the low and high dose groups. The incidence of granulomatous inflammation was mild and mild to severe in both groups. At the end of the recovery period (D71), granulomatous inflammation could still be seen in the muscles of the animals in the low and high dose groups. The degree of lesions was mild and mild to moderate, respectively, and the degree of lesion was alleviated, suggesting that there was a recovery trend. The lesions were considered as local irritant reactions caused by the aluminum hydroxide component in the vaccine. No obvious abnormal changes were found in other tissues and organs under the microscope. The histopathological report is shown in Table S13 and Figure 5B, C.

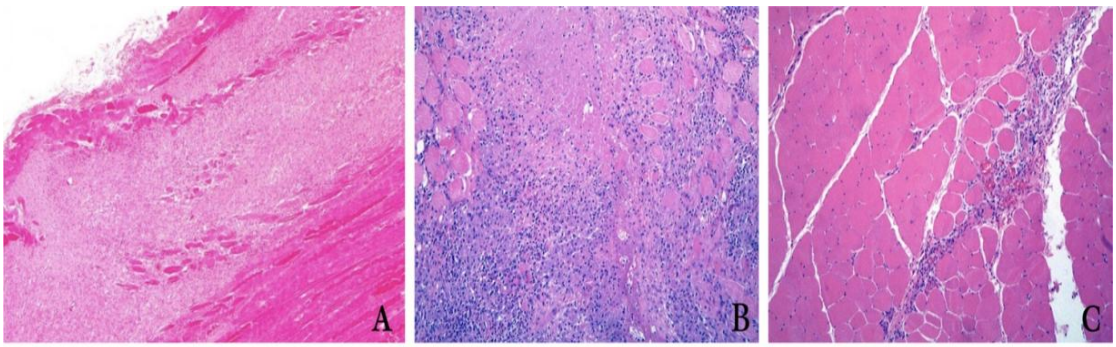

Figure 5. The results of the histopathological examination

(A) Animal No. 2120798 (SD rat, female) from the test sample group developed local granulomatous inflammation with a few inflammatory cell infiltrates after the drug administration (HE staining, 4X objective) (B) Animal No. 2120636 (cynomolgus monkey, female) from the negative control group was euthanized 3 days after the last drug (D46), and slight hemorrhage, muscle fiber necrosis and inflammatory cell infiltration were seen in the muscles at the injection site, focal (HE staining, 10X objective). (C) Animal No. 2120657 (cynomolgus monkey, female) from the high dose group were euthanized 3 days after the last dose (D46), and severe granulomatous inflammation, multifocal, was observed in the muscles of the injection site (HE staining, 10X objective).

Discussion

The best and most cost-efficient way to prevent and manage many infectious diseases is through vaccination[29-31]. The development of a vaccine centers on making sure it works and is safe for administration to healthy people. One of the most crucial steps in creating innovative vaccines is conducting a thorough preclinical safety evaluation [32, 33]. Preclinical safety evaluation of SARS-CoV-2 inactivated vaccine has not yet been performed [24, 34, 35], and methods for assessing the vaccine's safety are still being investigated.

Repeated dose toxicity studies using a single closely related animal species are commonly considered sufficient for vaccination products [36]. Test animals should have an immunological response and an effect on the immune system comparable to that of any adjuvant. SD rats and cynomolgus monkeys were utilized in a preclinical study to predict the toxicity of the SARS-CoV-2 inactivated vaccine to humans [37, 38]. Both animal species serve as useful models for the regulation of toxicity and

have prior research and experience. The inactivated vaccine induced specific immune responses and protective effects on rats and monkeys[39]. After repeated administration, a high level of specific IgG antibody against the S1 protein was detected in cynomolgus monkeys, indicating strong immunogenicity. No deaths or near deaths were recorded for SD rats injected once intramuscularly. Observations, weight, and food intake showed no abnormalities.

The results for the immunotoxicity indexes at different levels showed that there were no abnormal manifestations related to vaccine administration, such as allergic reactions, T lymphocyte subsets and globulin. The changes in serum cytokines in the cynomolgus monkeys were mainly characterized by decreased of TNF- α and IL-4 levels and increased IL-2, IL-5, and IL-6 levels. The decrease of the above-mentioned indexes TNF- α, IL-4 and the increase of IL-2 and IL-5 were considered to be of non-toxic significance because there was no obvious dose- response correlation or consistency between male and female animals. The increase of IL-6 combined with individual data was compared with the first administration (D-2) and the same period in the negative control group. Some animals in the low and high dose groups showed a slight increase in IL-6 after the first or last administration. The slight increase of IL-6 might be related to an acute phase reaction and/or an immune response induced by the vaccine[40]; however, the overall change was slight.

Three days after the first administration (D4), AST and Urea increased in the low dose group, K + decreased in the high dose group, and Glu and Ca decreased in the high dose group 3 days after the last administration (D46). There were no obvious dose-response correlations and/or time- response correlations, or consistency between males and females, and some of the indexes have no obvious change compared with their own pre-drug value (D-2); therefore, we considered these changes to be of non-toxic significance.

The results of gross anatomical observation showed that white nodules could be seen at the injection site of the SD rats in the test group, and mild/moderate granulomatous inflammation could be seen under the microscope. At the end of administration (D46), the probability of granulomatous inflammation in local muscle of cynomolgus monkeys in the low and high dose groups was three and six times higher than that of the control, respectively. And euthanasia at the end of recovery period (D71), Granulomatous inflammation can still be seen in the muscles at the injection site of the animals in the low-dose and high-dose groups (1 dose, 5 doses per animal), and the incidence rate is 100%. Granulomatous inflammation could still be seen in the muscle of animals in the low and high dose group, and the incidence rate was 44.6%.

Immune system toxicity, such as hypersensitivity and autoimmune disease, is a possible side effect of the vaccine[41]. Immunotoxicology focuses primarily on the study of autoimmunity, particularly as it relates to the use of adjuvant vaccinations [42]. The above changes were only seen in the test group and the incidence was high, which was considered to be related to the sample itself. Therefore, we believe that the changes observed under the microscope are the result of the adjuvant component (Al (OH)3) contained in the vaccine.

Some index changes that might be related to the tested products were observed after the animals were injected with the vaccine. At 4–6 hours after the last dose (D43), the body temperature of the male cynomolgus monkeys in the high dose group increased significantly ($P \leq 0.05$). Individual data showed that the body temperature of male cynomolgus monkeys was ≥ 40 ℃ in the low and high dose groups. Three days after the last dose (D46), the Eos of female cynomolgus monkeys increased in the high dose group, while the Mono (%) decreased significantly in the low and high dose groups. Three days after the first and last dose (D4, D46), the FIB of cynomolgus monkeys in the low and high dose groups showed an overall upward trend. The IL-6 of some cynomolgus monkeys in the low and high dose groups increased slightly after the first or last dose. The above changes mainly occurred after the first or last dose. Combined with the results of animal histopathological examination, granulomatous inflammation could be seen in the local injection site of the low and high dose groups, but no damage of lymphoid organs and tissues or other organs was found. This might be related to the acute phase reaction and/or vaccine-induced immune reaction and local irritation caused by the vaccine, and no abnormal changes were found in the recovery period. At the same time, the increase in Eos had a certain dose-response and time-response correlation, which might be related to the immune response induced by the vaccine. The change of Mono (%) was considered to be of non-toxic significance because there was no regular change, such as consistency between males and females. In addition, the extension of APTT was only seen in male animals, the range of change was small, and the average value is still within the reference range (the confidence interval of 95% of normal value of APTT in male animals is 15.02–44.95s), which is considered to be non-toxic.

We have also tested other SARS-CoV-2 variants, including the Delta strain, BA.2.3, BA.5.2.1, and XBB.1.5. The experimental results demonstrate that this vaccine exhibits strong protective efficacy against various COVID-19 variants[43].

This study still has several limitations. In the animal safety evaluation of the vaccine, animal models can typically only simulate certain aspects of the disease and cannot

fully capture the complexity of human diseases. Additionally, the immune response generated after administration did not include data on spleen immunity. Although the primary focus of this study was to evaluate vaccine safety, it would be beneficial to include results that reflect the protective efficacy of the vaccine.

To sum up, there was no obvious systemic toxicity when the vaccine was injected intramuscularly into cynomolgus monkeys and SD rats. The non-obvious toxic response dose of cynomolgus monkeys was 5 doses per monkey, and the rats had good humoral immune responses at 4 doses per rat. These results could provide valuable information for understanding the safety and efficacy of the vaccine and for designing future clinical trials

Ethics

The use of experimental animals in this study was approved by the Beijing Municipal Commission of Science and Technology (Approval Number: ACU21-2368).

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author Contributions

Z.W.H, J.L., J.L., H.M., Y.J.Z, Z.W., K.D.C., Y.L.L. conceived the study and designed experimental approaches. Z.W.H, J.L., J.L., H.M., Y.S., M.L.C., Z.B.F., F.B.X., L.H.W., C.L., J.X.L., Y.J.Z., Z.W., K.D.C. performed, analyzed, and interpreted the experiments. Z.W.H, J.L., H.M., Y.S., M.L.C., F.B.X., L.H.W., C.L., Y.J.Z, Z.W. contributed unique reagents. J.L., M.L.C., F.B.X. L.H.W. C.L. performed statistical analysis.Z.W.H, J.L., H.M., Z.B.F. wrote the manuscript with inputs from others. Every author has read, edited, and approved the final manuscript

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References

[1] Corman VM, Lienau J, Witzenrath M. Coronaviruses as the cause of respiratory infections[J]. Der Internist, 2019, 60(11): 1136.

[2] Kadam SB, Sukhramani GS, Bishnoi P, et al. SARS-CoV-2, the pandemic coronavirus: Molecular and structural insights[J]. Journal of basic microbiology, 2021, 61(3): 180-202.

[3] Yesudhas D, Srivastava A, Gromiha MM. COVID-19 outbreak: history, mechanism, transmission, structural studies and therapeutics[J]. Infection, 2021, 49(2): 199-213.

[4] Zheng XS, Wang Q, Xie T, et al. Assessment and sero-diagnosis for coronaviruses with risk of human spillover[J]. Emerging Microbes & Infections, 2023, 12(2): 2225932.

[5] Hoenigsperger H, Sivarajan R, Sparrer KMJ. Differences and similarities between innate immune evasion strategies of human coronaviruses[J]. Current Opinion in Microbiology, 2024, 79: 102466.

[6] Zaidi AK, Singh RB. Epidemiology of COVID-19. Prog Mol Biol Transl Sci. 2024;202:25-38.

[7] Meo SA, Bukhari IA, Akram J, et al. COVID-19 vaccines: comparison of biological, pharmacological characteristics and adverse effects of Pfizer/BioNTech and Moderna Vaccines[J]. European Review for Medical & Pharmacological Sciences, 2021, 25(3).

[8] Srivastava K, Carreño JM, Gleason C, et al. SARS-CoV-2-infection-and vaccine-induced antibody responses are long lasting with an initial waning phase followed by a stabilization phase[J]. Immunity, 2024, 57(3): 587-599. e4.

[9] Chalupka A, Richter L, Chakeri A, et al. Effectiveness of a fourth SARS-CoV-2 vaccine dose in previously infected individuals from Austria[J]. European Journal of Clinical Investigation, 2024, 54(3): e14136.

[10] Mirzakhani M, Bayat M, Dashti M, et al. The Assessment of Anti–SARS-CoV-2 Antibodies in Different Vaccine Platforms: A Systematic Review and Meta-Analysis of COVID-19 Vaccine Clinical Trial Studies[J]. Reviews in Medical Virology, 2024, 34(6): e2579.

[11] Li C, Guo Y, Fang Z, et al. Analysis of the protective efficacy of approved COVID-19 vaccines against various mutants[J]. Frontiers in Immunology, 2022, 13: 804945.

[12] Zedan HT, Smatti MK, Al-Sadeq DW, et al. SARS-CoV-2 infection triggers more potent antibody-dependent cellular cytotoxicity (ADCC) responses than mRNA-, vector-, and inactivated virus-based COVID-19 vaccines[J]. Journal of Medical Virology, 2024, 96(3): e29527.

[13] Saied ARA, Metwally AA, Alobo M, et al. Bovine-derived antibodies and camelid-derived nanobodies as biotherapeutic weapons against SARS-CoV-2 and its variants: a review article[J]. International Journal of Surgery, 2022, 98: 106233.

[14] Edara VV, Pinsky BA, Suthar MS, et al. Infection and vaccine-induced neutralizing-antibody responses to the SARS-CoV-2 B. 1.617 variants[J]. New England Journal of Medicine, 2021, 385(7): 664-666.

[15] Li XN, Huang Y, Wang W, et al. Effectiveness of inactivated SARS-CoV-2 vaccines against the Delta variant infection in Guangzhou: a test-negative case–control real-world study[J]. Emerging microbes & infections, 2021, 10(1): 1751-1759.

[16] Lopez Bernal J, Andrews N, Gower C, et al. Effectiveness of Covid-19 vaccines against the B. 1.617. 2 (Delta) variant[J]. New England Journal of Medicine, 2021, 385(7): 585-594.

[17] Pouwels KB, Pritchard E, Matthews PC, et al. Effect of Delta variant on viral burden and vaccine effectiveness against new SARS-CoV-2 infections in the UK[J]. Nature medicine, 2021, 27(12): 2127-2135.

[18] Martín Pérez C, Aguilar R, Jiménez A, et al. Correlates of protection and determinants of SARS-CoV-2 breakthrough infections 1 year after third dose vaccination[J]. BMC medicine, 2024, 22(1): 103.

[19] Chen Y, Liu Q, Zhou L, et al. Emerging SARS-CoV-2 variants: Why, how, and what's next?[J]. Cell Insight, 2022, 1(3): 100029.

[20] Lee M, Major M, Hong H. Distinct conformations of SARS-CoV-2 Omicron spike protein and its interaction with ACE2 and antibody[J]. International Journal of Molecular Sciences, 2023, 24(4): 3774.

[21] Ma C, Chen X, Mei F, et al. Drastic decline in sera neutralization against SARS-CoV-2 Omicron variant in Wuhan COVID-19 convalescents[J]. Emerging microbes & infections, 2022, 11(1): 567-572.

[22] Zhang NN, Zhang RR, Zhang YF, et al. Rapid development of an updated mRNA vaccine against the SARS-CoV-2 Omicron variant[J]. Cell research, 2022, 32(4): 401-403. •

[23] McMenamin ME, Nealon J, Lin Y, et al. Vaccine effectiveness of one, two, and three doses of BNT162b2 and CoronaVac against COVID-19 in Hong Kong: a population-based observational study[J]. The Lancet Infectious Diseases, 2022, 22(10): 1435-1443.

[24] Abdoli A, Aalizadeh R, Aminianfar H, et al. Safety and potency of BIV1-CovIran inactivated vaccine candidate for SARS-CoV-2: A preclinical study[J]. Reviews in medical virology, 2022, 32(3): e2305.

[25] Greenhouse DD. Institute of Laboratory Animal Resources. NATIONAL RESEARCH COUNCIL WASHINGTON DC COMMISSION ON LIFE SCIENCES; 1992.

[26] Act AW. United States Department of Agriculture (USDA) 2017 [Available from: https://www.nal.usda.gov/animal-health-and-welfare/animal-welfare-act.

[27] Kawataki M, Kashima T, Toda H, et al. Relation between QT interval and heart rate. Applications and limitations of Bazett's formula[J]. Journal of electrocardiology, 1984, 17(4): 371-375.

[28] Underwood W, Anthony R. AVMA guidelines for the euthanasia of animals: 2020 edition[J]. Retrieved on March, 2020, 2013(30): 2020-1.

[29] Philipson T. Economic epidemiology and infectious diseases[J]. Handbook of health economics, 2000, 1: 1761-1799.

[30] National Center for Infectious Diseases (US). Addressing emerging infectious disease threats: a prevention strategy for the United States[M]. Centers for Disease Control and Prevention, 1994.

[31] Connolly MA, Gayer M, Ryan MJ, et al. Communicable diseases in complex emergencies: impact and challenges[J]. The Lancet, 2004, 364(9449): 1974-1983.

[32] Lebron JA, Wolf JJ, Kaplanski CV, et al. Ensuring the quality, potency and safety of vaccines during preclinical development[J]. Expert review of vaccines, 2005, 4(6): 855-866.

[33] Stern PL. Key steps in vaccine development[J]. Annals of Allergy, Asthma & Immunology, 2020, 125(1): 17-27.

[34] Huang Z, Jiang Q, Wang Y, et al. SARS-CoV-2 inactivated vaccine (Vero cells) shows good safety in repeated administration toxicity test of Sprague Dawley rats[J]. Food and Chemical Toxicology, 2021, 152: 112239.

[35] Ghasemi S, Naderi Saffar K, Ebrahimi F, et al. Development of inactivated FAKHRAVAC® vaccine against SARS-CoV-2 virus: preclinical study in animal models[J]. Vaccines, 2021, 9(11): 1271.

[36] Forster R. Study designs for the nonclinical safety testing of new vaccine products[J]. Journal of pharmacological and toxicological methods, 2012, 66(1): 1-7.

[37] Mancebo A, Casaco A, Sanchez B, et al. Repeated dose (14 days) rat intramuscular toxicology study of Her1 vaccine[J]. Regulatory Toxicology and Pharmacology, 2012, 64(3): 425-434.

[38] Wolf JJ, Kaplanski CV, Lebron JA. Nonclinical safety assessment of vaccines and adjuvants[J]. Vaccine Adjuvants: Methods and Protocols, 2009: 29-40.

[39] Poria R, Kala D, Nagraik R, et al. Vaccine development: Current trends and technologies[J]. Life Sciences, 2024, 336: 122331.

[40] Khalil RH, Al-Humadi N. Types of acute phase reactants and their importance in vaccination[J]. Biomedical reports, 2020, 12(4): 143-152.

[41] Henson KL. Preclinical Evaluation of Immunotoxicity[J]. Schalm's Veterinary Hematology, 2022: 116-121.

[42] Guimarães LE, Baker B, Perricone C, et al. Vaccines, adjuvants and autoimmunity[J]. Pharmacological research, 2015, 100: 190-209.

[43] Zhang Y, Mao H, Li J, et al. Delta (B. 1.617. 2) inactivated vaccine candidate elicited neutralizing antibodies to SARS-CoV-2 and circulating variants in rhesus macaques[J]. Virologica Sinica, 2023, 38(4): 627-630.

Supplementary Tables

Table S1 Electrocardiogram parameter statistics in cynomolgus monkey (Mean ± SD）

Group	Day(s) Relative to Start Date	Hheart rate (/Min)	PR Interval (Seconds)	QT Interval (Seconds)	QRS duration (Seconds)	QTcB (Seconds)
Group 1 (Male)	-2 (N=5)	257.6 ± 38.8	0.058 ± 0.004	0.158 ± 0.008	0.050 ± 0.000	0.326 ± 0.016
	1 (N=5)	267.4 ± 25.4	0.056 ± 0.005	0.164 ± 0.015	0.050 ± 0.000	0.345 ± 0.015
	2 (N=5)	266.2 ± 32.7	0.056 ± 0.009	0.154 ± 0.005	0.050 ± 0.000	0.323 ± 0.010
	43 (N=5)	274.8 ± 32.6	0.056 ± 0.005	0.154 ± 0.009	0.050 ± 0.000	0.328 ± .0.13
	44 (N=5)	274.6 ± 23.9	0.056 ± 0.005	0.154 ± 0.009	0.050 ± 0.000	0.329 ± 0.018
	70 (N=2)	270.5 ± 12.0	0.060 ± 0.000	0.155 ± 0.007	0.050 ± 0.000	0.329 ± 0.008
Group 1 (Female)	-2 (N=5)	253.4 ± 29.2	0.054 ± 0.009	0.164 ± 0.021	0.050 ± 0.000	0.335 ± 0.023
	1 (N=5)	242.4 ± 37.8	0.056 ± 0.009	0.166 ± 0.015	0.050 ± 0.000	0.331 ± 0.009
	2 (N=5)	245.8 ± 29.2	0.056 ± 0.009	0.164 ± 0.015	0.050 ± 0.000	0.330 ± 0.019
	43 (N=5)	257.2 ± 25.1	0.058 ± 0.008	0.162 ± 0.011	0.048 ± 0.004	0.334 ± 0.013
	44 (N=5)	257.0 ± 23.0	0.058 ± 0.004	0.166 ± 0.013	0.050 ± 0.000	0.342 ± 0.015
	70 (N=2)	247.5 ± 17.7	0.065 ± 0.021	0.160 ± 0.000	0.050 ± 0.000	0.325 ± 0.012
Group 2 (Male)	-2 (N=5)	243.8 ± 31.1	0.054 ± 0.005	0.158 ± 0.009	0.050 ± 0.000	0.313 ± 0.012
	1 (N=5)	234.6 ± 31.6	0.062 ± 0.004	0.160 ± 0.000	0.050 ± 0.000	0.316 ± 0.022
	2 (N=5)	240.0 ± 28.4	0.058 ± 0.004	0.162 ± 0.011	0.050 ± 0.000	0.323 ± 0.020
	43 (N=5)	251.0 ± 28.3	0.054 ± 0.005	0.154 ± 0.009	0.050 ± 0.000	0.314 ± 0.014
	44 (N=5)	242.4 ± 23.7	0.060 ± 0.010	0.156 ± 0.011	0.050 ± 0.000	0.313 ± 0.018
	70 (N=2)	244.0 ± 41.0	0.055 ± 0.007	0.150 ± 0.014	0.045 ± 0.007	0.301 ± 0.003

Group 2 (Female)	-2 (N=5)	259.2 ± 32.1	0.054 ± 0.005	0.162 ± 0.011	0.050 ± 0.000	0.335 ± 0.015
	1 (N=5)	262.6 ± 24.7	0.052 ± 0.004	0.160 ± 0.007	0.046 ± 0.005	0.334 ± 0.010
	2 (N=5)	252.2 ± 23.4	0.056 ± 0.005	0.166 ± 0.013	0.044* ± 0.005	0.340 ± 0.024
	43 (N=5)	257.0 ± 28.6	0.058 ± 0.008	0.162 ± 0.013	0.050 ± 0.000	0.334 ± 0.009
	44 (N=5)	254.6 ± 24.0	0.058 ± 0.008	0.168 ± 0.013	0.048 ± 0.004	0.345 ± 0.016
	70 (N=2)	277.0 ± 9.9	0.055 ± 0.007	0.155 ± 0.007	0.045 ± 0.007	0.333 ± 0.009
Group 3 (Male)	-2 (N=5)	279.2 ± 21.3	0.054 ± 0.005	0.156 ± 0.005	0.048 ± 0.004	0.336 ± 0.008
	1 (N=5)	271.8 ± 21.4	0.058 ± 0.004	0.152 ± 0.004	0.050 ± 0.000	0.323 ± 0.016
	2 (N=5)	278.4 ± 25.3	0.052 ± 0.004	0.160 ± 0.000	0.048 ± 0.004	0.344 ± 0.016
	43 (N=5)	177.6 ± 18.6	0.060 ± 0.007	0.156 ± 0.009	0.050 ± 0.000	0.335 ± 0.013
	44 (N=5)	273.0 ± 28.5	0.064 ± 0.005	0.158 ± 0.018	0.050 ± 0.000	0.335 ± 0.023
	70 (N=2)	249.0 ± 31.1	0.060 ± 0.000	0.160 ± 0.000	0.050 ± 0.000	0.326 ± 0.020
Group 3 (Female)	-2 (N=5)	240.8 ± 32.2	0.054 ± 0.005	0.166 ± 0.019	0.050 ± 0.000	0.330 ± 0.023
	1 (N=5)	250.4 ± 22.0	0.056 ± 0.005	0.160 ± 0.007	0.050 ± 0.000	0.326 ± 0.011
	2 (N=5)	251.2 ± 7.9	0.056 ± 0.005	0.162 ± 0.004	0.050 ± 0.000	0.331 ± 0.007
	43 (N=5)	263.4 ± 9.8	0.056 ± 0.005	0.160 ± 0.000	0.048 ± 0.004	0.335 ± 0.006
	44 (N=5)	266.4 ± 6.4	0.060 ± 0.000	0.162 ± 0.011	0.048 ± 0.004	0.341 ± 0.021
	70 (N=2)	243.0 ± 14.1	0.055 ± 0.007	0.160 ± 0.000	0.050 ± 0.000	0.322 ± 0.009

Note: “N” was cynomolgus monkey number of each group.

Table S2. Statistical data of urine routine examination of cynomolgus monkey

Group	Day(s) Relative to Start Date	BLD	LEU	NIT	GLU	PRO	BIL	RBC	WBC	pH	BAC
Group 1(Male)	-1 (N=5)	3+, 1/5; 1+, 1/5; Neg, 1/5; 2+, 1/5; 4+, 1/5	1+, 2/5; Neg, 3/5	Neg, 4/5; Pos, 1/5	Neg, 5/5	1+, 4/5; Neg, 1/5	Neg, 4/5; 1+, 1/5	1.14, 5/5	2.25, 1/5; 1.14, 4/5	9, 4/5; 8, 1/5	3+, 3/5; 2+, 1/5; Neg, 1/5
	45 (N=5)	1+, 1/5; 3+, 2/5; 4+, 2/5	Neg, 5/5	Neg, 5/5	Neg, 5/5	Neg, 4/5; 1+, 1/5	Neg, 5/5	1.14, 5/5	1.14, 5/5	9, 4/5; 8, 1/5	1+, 1/5; 2+, 3/5; 3+, 1/5
	70 (N=5)	1+, 1/2; 4+, 1/2	Neg, 1/2; 1+, 1/2	Neg, 2/2	Neg, 2/2	Neg, 1/2; 1+, 1/2	Neg, 2/2	1.14, 2/2	1.14, 2/2	8, 2/2	1+, 1/2; 2+, 1/2
Group1(Female)	-1 (N=5)	5+, 5/5	1+, 2/5; 3+, 2/5; neg, 1/5	Neg, 4/5; pos, 1/5	Neg, 4/5; 1+, 1/5	1+, 3/5; 2+, 2/5	Neg, 5/5	1.14, 3/5; 18.00, 1/5; 68.60, 1/5	1.14, 3/5; 5.40, 1/5; 20.40, 1/5	8, 4/5; 7, 1/5	Neg, 4/5; 3+, 1/5
	45 (N=5)	2+, 1/5;3+, 1/5; 4+, 1/5; 5+, 2/5	1+, 1/5; 2+, 2/5; 3+, 2/5	Neg, 4/5; Pos, 1/5	Neg, 5/5	Neg, 2/5; 1+, 3/5	Neg, 5/5	1.14, 5/5	1.14, 5/5	8, 5/5	Neg, 4/5; 1+, 1/5
	70 (N=5)	1+, 1/2; 5+, 1/2	2+, 1/2; 3+, 1/2	Neg, 2/2	Neg, 2/2	Neg, 1/2; 1+, 1/2	Neg, 2/2	1.14, 2/2	1.14, 2/2	8, 2/2	Neg, 1/2; 1+, 1/2
Group 2(Male)	-1 (N=5)	3+, 2/5; 1+, 2/5; 4+, 1/5	Neg, 4/5; 1+, 1/5	Neg, 5/5	Neg, 5/5	Neg, 2/5; 1+, 3/5	Neg, 5/5	1.14, 5/5	1.14, 5/5	9, 3/5; 8, 2/5	2+, 3/5; 1+, 1/5; Neg, 1/5
	45 (N=5)	2+, 1/5;3+, 2/5; 4+, 1/5; 5+, 1/5	1+, 1/5; 2+, 1/5; Neg, 3/5	Neg, 4/5; Pos, 1/5	Neg, 5/5	Neg, 4/5; 1+, 1/5	Neg, 5/5	1.14, 5/5	1.14, 5/5	9, 3/5; 8, 2/5	1+, 1/5; 2+, 2/5; 3+, 2/5
	70 (N=5)	4+, 1/2;5+, 1/2	1+, 1/2; 2+,1/2	Neg, 2/2	Neg, 2/2	Neg, 2/2	Neg, 2/2	1.14, 2/2	1.14, 2/2	8, 2/2	2+, 2/2

Group2(Female)	-1 (N=5)	2+, 2/5; 3+, 1/5; 4+, 1/5; 5+, 1/5	1+, 1/5; 2+, 1/5; 3+, 1/5; Neg, 2/5	Neg, 5/5	Neg, 5/5	Neg, 3/5; 1+, 2/5	Neg, 5/5	1.14, 5/5	1.14, 5/5	9, 4/5; 8,1/5	1+, 1/5; 2+, 2/5; 3+, 1/5; Neg, 1/5
	45 (N=5)	2+, 1/5; 3+, 1/5; 4+, 2/5; 5+, 1/5	3+, 5/5	Neg, 5/5	Neg, 5/5	Neg, 1/5; 1+, 3/5; 2+, 1/5	Neg, 5/5	1.14, 5/5	1.14, 2/5; 1.88, 1/5; 6.30, 1/5; 45.45, 1/5	8, 5/5	1+, 1/5; 2+, 3/5; Neg, 1/5
	70 (N=5)	3+, 1/2; 5+, 1/2	3+, 2/2	Neg, 2/2	Neg, 2/2	1+, 2/2	Neg, 2/2	1.14, 2/2	1.14, 2/2	8, 2/2	Neg, 1/2; 2+, 1/2
Group 3(Male)	-1 (N=5)	2+, 1/5; 3+, 1/5; 5+, 1/5; Neg, 2/5	Neg, 4/5;1+, 1/5	Neg, 5/5	Neg, 5/5	Neg, 2/5; 1+, 2/5; 2+, 1/5	Neg, 5/5	1.14, 5/5	1.14, 5/5	9, 4/5; 8, 1/5	1+, 2/5;2+, 2/5; 3+, 1/5
	45 (N=5)	1+, 1/5; 4+, 1/5; 5+, 2/5; Neg, 1/5	Neg, 4/5; 2+, 1/5	Neg, 3/5; Pos, 2/5	Neg, 5/5	Neg, 1/5; 1+, 4/5	Neg, 5/5	1.14, 5/5	1.14, 4/5; 1.80, 1/5	9, 4/5; 8, 1/5	2+, 2/5; 3+, 2/5; Neg, 1/5
	70 (N=5)	2+, 1/2; 5+, 1/2	Neg, 2/2	Neg, 2/2	Neg, 2/2	Neg, 1/2; 1+, 1/2	Neg, 2/2	1.14, 2/2	1.14, 2/2	8, 2/2	1+, 1/2; 2+, 1/2
Group3(Female)	-1 (N=5)	1+, 2/5; 4+, 1/5;5+, 2/5	1+, 1/5; 3+, 2/5; neg, 2/5	Neg, 5/5	Neg, 5/5	Neg, 4/5; 1+, 1/5	Neg, 5/5	1.14, 4/5; 9.00, 1/5	1.14, 4/5; 1.80, 1/5	9, 3/5; 8, 2/5	1+, 1/5; 2+, 1/5; 3+, 1/5; Neg, 2/5
	45 (N=5)	3+, 1/5; 4+, 1/5; 5+, 3/5	1+, 1/5; 2+, 4/5	Neg, 3/5; Pos, 2/5	Neg, 5/5	Neg, 1/5; 1+, 4/5	Neg, 5/5	1.14, 5/5	1.14, 5/5	9, 1/5; 8, 4/5	1+, 1/5; 2+, 2/5; Neg, 2/5
	70 (N=5)	2+, 1/2; 5+, 1/2	3+, 1/2	Neg, 2/2	Neg, 2/2	Neg, 1/2; 1+, 1/2	Neg, 2/2	1.14, 2/2	1.14, 1/2; 45.90, 1/2	8, 2/2	Neg, 2/2

Table S3. Statistical data of hematology examination in cynomolgus monkey

Group	Day(s) Relative to Start Date	WBC (10^9/L)	Neut (%)	Lymph (%)	Mono (%)	Eos (%)	Baso (%)	LUC (%)	RBC (10^{12}/L)	HGB (g/L)	HCT (%)	Retic (%)
Group1 (Male)	-2(N=5)	13.652 ± 1.241	44.60 ± 7.91	47.80 ± 8.27	4.90 ± 1.64	1.32 ± 0.83	0.50 ± 0.07	0.86 ± 0.21	5.176 ± 0.353	128.6 ± 9.9	43.28 ± 2.33	0.0650 ± 0.0184
	4(N=5)	12.138 ± 1.586	48.18 ± 8.36	43.48 ± 8.85	5.32 ± 2.07	1.38 ± 0.63	0.60 ± 0.23	1.02 ± 0.63	4.990 ± 0.328	127.6 ± 7.4	41.12 ± 2.15	1.398 ± 0.512
	46(N=5)	12.232 ± 3.091	48.46 ± 6.05	44.76 ± 6.55	4.22 ± 0.72	1.36 ± 1.22	0.40 ± 0.07	0.80 ± 0.16	5.402 ± 0.287	131.4 ± 8.8	43.78 ± 3.68	1.828 ± 0.544
	71(N=2)	12.020 ± 1.655	47.80 ± 1.84	44.50 ± 3.82	5.10 ± 3.25	1.60 ± 0.99	0.40 ± 0.14	0.65 ± 0.21	5.670 ± 0.198	130.0 ± 5.7	45.70 ± 1.84	0.916 ± 0.208
Group1 (Female)	-2(N=5)	12.078 ± 6.258	48.70 ± 19.12	43.44 ± 17.85	4.28 ± 0.59	2.44 ± 2.99	0.44 ± 0.28	0.68 ± 0.38	5.216 ± 0.443	128.2 ± 12.8	41.30 ± 3.40	1.708 ± 0.231
	4(N=5)	10.960 ± 2.281	42.00 ± 8.36	48.30 ± 10.29	5.02 ± 1.76	3.66 ± 2.83	0.38 ± 0.08	0.72 ± 0.15	4.724 ± 0.282	120.2 ± 11.9	38.86 ± 2.36	1.594 ± 0.705
	46(N=5)	12.004 ± 5.133	47.90 ± 13.23	42.16 ± 12.71	6.36 ± 0.75	2.44 ± 1.75	0.42 ± 0.13	0.72 ± 0.23	5.002 ± 0.779	116.8 ± 22.5	39.14 ± 6.66	1.282 ± 1.000
	71(N=2)	13.035 ± 1.704	49.45 ± 9.12	44.10 ± 9.62	3.90 ± 0.85	1.25 ± 0.49	0.35 ± 0.07	1.00 ± 0.28	5.595 ± 0.02	132.0 ± 8.5	46.40 ± 4.10	1.255 ± 0.247
Group2 (Male)	-2(N=5)	10.504 ± 3.502	53.08 ± 1.53	39.48 ± 0.98	4.42 ± 1.18	2.16 ± 0.62	0.30* ± 0.07	0.52 ± 0.15	5.246 ± 0.108	129.6 ± 5.1	42.38 ± 2.16	0.0560 ± 0.0170
	4(N=5)	10.202 ± 4.991	54.24 ± 8.22	37.66 ± 8.27	4.60 ± 1.10	2.36 ± 0.68	0.36 ± 0.05	0.74 ± 0.35	5.120 ± 0.242	130.8 ± 6.5	41.48 ± 2.28	1.142 ± 0.252
	46(N=5)	13.960 ± 3.928	52.46 ± 21.95	38.26 ± 19.78	4.50 ± 0.71	3.50 ± 2.15	0.54 ± 0.23	0.70 ± 0.19	5.746 ± 0.295	137.6 ± 7.2	45.30 ± 2.64	1.984 ± 0.574
	71(N=2)	7.460 ± 1.782	40.80 ± 19.94	50.50 ± 23.05	4.85 ± 3.46	2.20 ± 1.41	0.40 ± 0.14	1.20 ± 0.85	6.095 ± 0.233	142.5 ± 9.2	49.30 ± 1.41	0.906 ± 0.237

Group2 (Female)	-2(N=5)	11.836 ± 2.502	46.76 ± 6.06	44.54 ± 6.85	5.10 ± 2.69	2.34 ± 1.16	0.46 ± 0.05	0.80 ± 0.16	5.166 ± 0.234	127.0 ± 5.2	40.86 ± 1.27	1.500 ± 0.609
	4(N=5)	12.886 ± 3.196	51.26 ± 4.18	41.48 ± 4.83	4.14 ± 1.44	2.04 ± 0.64	0.40 ± 0.07	0.68 ± 0.18	4.876 ± 0.272	123.6 ± 6.8	39.64 ± 1.45	1.686 ± 0.987
	46(N=5)	12.054 ± 2.216	56.00 ± 8.63	36.16 ± 9.19	4.82* ± 1.25	2.18 ± 0.92	0.40 ± 0.10	0.48 ± 0.13	5.196 ± 0.406	121.8 ± 6.3	40.98 ± 1.77	1.104 ± 0.284
	71(N=2)	11.560 ± 1.853	49.05 ± 3.89	45.15 ± 3.18	3.50 ± 0.00	1.10 ± 0.57	0.40 ± 0.14	0.80 ± 0.00	5.215 ± 0.163	119.5 ± 0.7	43.50 ± 0.28	1.455 ± 0.247
Group3 (Male)	-2(N=5)	12.348 ± 5.023	43.88 ± 9.15	47.64 ± 9.85	5.56 ± 1.12	1.84 ± 0.84	0.42 ± 0.13	0.64 ± 0.23	5.302 ± 0.394	128.2 ± 6.4	41.44 ± 2.25	0.0535 ± 0.0078
	4(N=5)	9.604 ± 2.975	51.10 ± 7.42	39.52 ± 7.29	6.28 ± 0.66	1.90 ± 0.73	0.42 ± 0.16	0.78 ± 0.35	5.196 ± 0.257	129.0 ± 5.0	40.50 ± 1.18	1.112 ± 0.435
	46(N=5)	11.394 ± 2.188	50.64 ± 9.82	38.34 ± 10.77	6.28* ± 1.11	3.60 ± 1.66	0.46 ± 0.13	0.68 ± 0.20	5.914 ± 0.440	136.2 ± 9.2	44.82 ± 2.72	1.754 ± 0.475
	71(N=2)	9.830 ± 2.362	33.40 ± 5.80	58.25 ± 8.41	5.60 ± 1.56	1.30 ± 0.99	0.35 ± 0.07	1.10 ± 0.28	6.275 ± 0.728	141.5 ± 16.3	49.25 ± 5.44	0.854 ± 0.318
Group3 (Female)	-2(N=5)	11.504 ± 2.465	46.38 ± 16.46	45.96 ± 15.88	3.96 ± 0.81	2.70 ± 1.33	0.40 ± 0.19	0.58 ± 0.18	5.264 ± 0.397	129.0 ± 11.1	41.00 ± 2.76	1.962 ± 0.591
	4(N=5)	13.134 ± 4.660	53.92 ± 16.42	36.98 ± 15.07	6.00 ± 2.40	2.10 ± 0.79	0.54 ± 0.28	0.64 ± 0.21	4.992 ± 0.378	127.2 ± 9.9	39.12 ± 2.46	2.124 ± 0.794
	46(N=5)	14.398 ± 4.231	47.90 ± 12.14	41.34 ± 10.97	4.84* ± 0.50	4.66 ± 1.99	0.54 ± 0.05	0.70 ± 0.16	5.408 ± 0.425	125.8 ± 12.2	41.30 ± 3.44	1.444 ± 1.171
	71(N=2)	9.460 ± 1.018	43.80 ± 16.69	49.70 ± 14.14	3.25 ± 1.34	2.30 ± 0.99	0.20 ± 0.14	0.75 ± 0.07	5.255 ± 0.304	119.5 ± 2.1	42.15 ± 1.77	1.690 ± 0.255

Note: “N” was cynomolgus monkey number of each group. * = $p < 0.05$.

Table S4. Statistical data of blood coagulation function of cynomolgus monkeys

Group	Day(s) Relative to Start Date	PT (Seconds)	APTT (Seconds)	FIB (g/L)
Group 1 (Male)	-2 (n=5)	10.04±0.40	19.40±2.15	3.0304±0.2977
	4 (n=5)	9.86±0.30	19.40±2.97	3.4252±0.6286
	46 (n=5)	10.24±0.21	20.64±2.94	3.1414±0.4344
	71 (n=2)	9.70±0.14	20.05±0.78	2.9840±0.2150
Group 1 (Female)	-2 (n=5)	9.80±0.82	21.12±3.33	2.7384±0.7030
	4 (n=5)	9.70±0.45	21.34±3.22	2.4122±0.1680
	46 (n=5)	9.56±0.46	20.62±2.69	2.6922±0.5742
	71 (n=2)	9.65±0.21	20.20±0.14	2.1130±0.0849
Group 2 (Male)	-2 (n=5)	10.22±0.45	19.54±0.87	3.6696±0.5779
	4 (n=5)	9.88±0.44	19.64±1.34	3.9980±0.3808
	46 (n=5)	10.10±0.46	23.00±4.07	3.9994±1.9508
	71 (n=2)	10.10±0.42	21.35±0.64	2.9450±0.3903

Group 2 (Female)	-2 (n=5)	9.80±0.54	21.20±2.34	2.8556±0.3296
	4 (n=5)	9.90±0.58	24.28±4.70	3.2408±0.6110
	46 (n=5)	9.86±0.49	24.04±2.65	3.0420±0.4845
	71 (n=2)	9.40±0.28	23.80±2.83	2.4850±0.0000
Group 3 (Male)	-2 (n=5)	10.30±0.37	21.56±2.18	3.3238±0.6528
	4 (n=5)	10.24±0.45	23.48±2.95	4.3036±0.7477
	46 (n=5)	10.40±0.37	23.42±1.23	2.9802±0.2586
	71 (n=2)	10.00±0.14	22.75±0.64	2.6095±0.0841
Group 3 (Female)	-2 (n=5)	9.98±0.28	19.32±1.09	2.8842±0.9400
	4 (n=5)	10.24±0.28	21.42±0.94	3.3962±0.8947
	46 (n=5)	9.88±0.33	21.08±1.00	3.5328±1.5184
	71 (n=2)	10.05±0.49	22.05±2.62	3.4380±1.1314

Note: “N” was cynomolgus monkey number of each group. * = $p < 0.05$.

Table S5. Statistical data of serum biochemistry of cynomolgus monkeys

Group	Day(s) Relative to Start Date	ALT (U/L)	AST (U/L)	ALP (U/L)	TBil (μmol/L)	DBil (μmol/L)	GGT (U/L)	TP (g/L)	Alb (g/L)	Glb (g/L)	Glu (mmol/L)	UREA (mmol/L)	TG (mmol/L)
Group1 (Male)	-2(N=5)	71.4 ± 19.5	48.2 ± 9.9	532.8 ± 138.5	2.284 ± 1.889	0.670 ± 0.137	67.2 ± 26.3	74.40 ± 5.57	46.04 ± 2.53	28.36 ± 3.87	6.094 ± 1.304	6.16 ± 0.71	0.408 ± 0.089
	4(N=5)	61.6 ± 8.9	36.2 ± 4.4	520.8 ± 164.6	2.170 ± 0.736	0.776 ± 0.103	60.2 ± 24.9	74.38 ± 6.23	43.98 ± 4.07	30.40 ± 3.49	4.866 ± 0.943	5.50 ± 0.65	0.412 ± 0.068
	46(N=5)	68.8 ± 19.0	51.6 ± 17.1	523.4 ± 143.1	1.886 ± 0.319	1.028 ± 0.176	66.2 ± 22.4	77.28 ± 5.80	49.34 ± 2.85	27.94 ± 4.57	4.704 ± 0.911	6.40 ± 0.90	0.374 ± 0.134
	71(N=2)	53.5 ± 13.4	55.0 ± 24.0	474.5 ± 36.1	0.935 ± 0.332	0.690 ± 0.198	47.5 ± 0.7	75.00 ± 3.96	42.35n ± 0.64	32.65 ± 4.60	5.665 ± 0.870	5.75 ± 0.92	0.345 ± 0.007
Group1 (Female)	-2(N=5)	66.6 ± 28.3	48.0 ± 17.2	321.4 ± 110.6	1.638 ± 0.907	0.758 ± 0.307	57.6 ± 18.0	72.80 ± 2.79	45.34 ± 2.12	27.46 ± 1.74	4.964 ± 1.694	6.04 ± 1.16	0.438 ± 0.195
	4(N=5)	116.2 ± 51.1	44.8 ± 8.6	348.2 ± 126.8	1.668 ± 0.433	0.738 ± 0.195	60.8 ± 18.5	69.90 ± 2.17	43.94 ± 1.75	25.96 ± 1.85	5.974 ± 1.188	5.72 ± 0.78	0.448 ± 0.235
	46(N=5)	67.6 ± 16.6	40.6 ± 9.8	281.6 ± 75.1	1.548 ± 1.049	0.746 ± 0.384	53.4 ± 19.7	68.44 ± 5.72	43.94 ± 6.63	24.50 ± 2.90	4.446 ± 0.383	6.62 ± 1.04	0.360 ± 0.109
	71(N=2)	45.5 ± 9.2	46.5 ± 19.1	316.5 ± 103.9	1.560 ± 0.085	0.685 ± 0.168	57.0 ± 15.6	71.40 ± 4.38	45.40 ± 0.99	26.00 ± 3.39	5.270 ± 0.212	5.60 ± 0.28	0.335 ± 0.049
Group2 (Male)	-2(N=5)	94.2 ± 92.7	134.4 ± 146.5	561.4 ± 137.5	1.744 ± 0.653	0.974 ± 0.410	69.4 ± 10.0	73.72 ± 5.68	45.46 ± 3.15	28.26 ± 3.77	4.762 ± 0.833	6.04 ± 0.68	0.394 ± 0.073
	4(N=5)	58.8 ± 19.2	52.8 ± 11.3	616.8 ± 92.3	1.714 ± 0.437	0.808 ± 0.319	61.4 ± 12.2	75.42 ± 5.16	44.28 ± 2.69	31.14 ± 3.92	4.208 ± 0.375	5.58 ± 0.54	0.508 ± 0.124
	46(N=5)	49.0 ± 13.8	46.4 ± 11.5	609.2 ± 140.3	1,362 ± 0,960	0.680 ± 0.527	78.0 ± 8.5	79.78 ± 4.41	48.36 ± 3.06	31.42 ± 5.39	4.108 ± 0.415	6.58 ± 1.22	0.458 ± 0.145
	71(N=2)	44.5 ± 16.3	46.0 ± 2.8	706.0 ± 53.7	0.790 ± 0.085	0.635 ± 0.120	84.0 ± 0.0	76.60 ± 1.84	46.10n ± 0.85	30.50 ± 0.99	5.115 ± 0.148	5.90 ± 0.71	0.510 ± 0.297

Group2 (Female)	-2(N=5)	57.4 ± 19.0	50.2 ± 19.8	352.8 ± 91.3	2.412 ± 0.357	1.060 ± 0.307	57.4 ± 16.9	75.10 ± 3.56	45.36 ± 3.11	28.74 ± 2.51	4.324 ± 0.629	6.12 ± 0.99	0.440 ± 0.099
	4(N=5)	65.2 ± 18.1	46.6 ± 13.1	373.8 ± 50.9	2.404 ± 0.579	1.062 ± 0.337	55.0 ± 18.1	**74.66*** ± 3.42	44.10 ± 2.76	30.56 ± 3.38	5.226 ± 0.991	5.82 ± 0.62	0.488 ± 0.119
	46(N=5)	70.0 ± 23.6	49.2 ± 14.1	316.2 ± 79.1	2.776 ± 1.377	1.450 ± 0.762	55.4 ± 16.9	74.92 ± 2.72	76.22 ± 2.67	28.70 ± 3.66	4.278 ± 0.243	6.30 ± 0.74	0.380 ± 0.072
	71(N=2)	66.5 ± 9.2	51.5 ± 0.7	271.0 ± 58.0	1.370 ± 0.778	0.410 ± 0.042	57.0 ± 9.9	69.65 ± 8.27	37.35 ± 7.28	32.30 ± 0.99	5.900 ± 1.725	6.75 ± 0.64	0.580n ± 0.057
Group3 (Male)	-2(N=5)	74.4 ± 23.5	63.6 ± 25.1	475.2 ± 172.9	1.686 ± 0.596	0.800 ± 0.161	69.8 ± 23.8	71.80 ± 6.33	44.86 ± 3.13	26.94 ± 0.98	5.324 ± 1.833	6.54 ± 0.44	0.348 ± 0.194
	4(N=5)	71.2 ± 8.7	**53.6*** ± 14.6	507.8 ± 178.8	2.198 ± 0.243	0.946 ± 0.110	59.8 ± 11.0	73.52 ± 1.84	42.54 ± 3.76	30.98 ± 2.49	4.242 ± 0.667	**6.62*** ± 0.78	0.390 ± 0.129
	46(N=5)	68.2 ± 13.3	57.6 ± 6.7	498.4 ± 174.3	2.430 ± 0.605	1.244 ± 0.450	70.0 ± 15.9	77.28 ± 1.99	49.44 ± 1.04	27.84 ± 1.56	3.992 ± 0.810	6.94 ± 0.72	0.448 ± 0.216
	71(N=2)	80.0 ± 5.7	70.5 ± 10.6	530.0 ± 209.3	0.835 ± 0.177	0.630 ± 0.170	81.5 ± 26.2	76.45 ± 7.14	49.20n ± 2.40	27.25 ± 9.55	4.945 ± 0.898	5.75 ± 0.07	0.315 ± 0.049
Group3 (Female)	-2(N=5)	78.2 ± 42.6	70.8 ± 58.4	475.0 ± 145,4	2.418 ± 0.819	1.004 ± 0.362	46.4 ± 15.5	70.66 ± 2.15	44.18 ± 4.57	28.46 ± 4.04	4.662 ± 0.720	6.88 ± 0.86	0.698 ± 0.601
	4(N=5)	71.6 ± 24.5	40.4 ± 9.2	481.2 ± 157.8	2.342 ± 0.950	0.864 ± 0.629	64.8 ± 13.1	71.30 ± 1.00	42.74 ± 3.76	28.56 ± 3.78	4.876 ± 0.689	6.20 ± 0.90	0.452 ± 0.112
	46(N=5)	59.8 ± 28.5	47.0 ± 9.1	431.0 ± 135.0	1.376 ± 0.794	0.720 ± 0.307	58.4 ± 15.8	68.88 ± 4.45	38.80 ± 10.29	30.08 ± 7.67	**3.928*** ± 0.133	6.08 ± 0.61	0.560 ± 0.343
	71(N=2)	53.5 ± 10.6	82.0 ± 14.1	502.0 ± 233.3	0.945 ± 0.078	0.670 ± 0.141	77.0 ± 0.0	68.75 ± 0.35	34.70 ± 7.35	34.05 ± 7.00	4.650 ± 0.467	5.25 ± 0.49	0.370n ± 0.057

Note: “N” was cynomolgus monkey number of each group. * = $p < 0.05$. n: inappropriate for statistics

Table S6. Statistical data of electrolyte of cynomolgus monkeys

Group	Day(s) Relative to Start Date	Ca (mmol/L)	P (mmol/L)	Na^+ (mmol/L)	K^+ (mmol/L)	Cl^- (mmol/L)
Group 1(Male)	-2(N=5)	2.762 ± 0.143	1.692 ± 0.347	146.06 ± 1.48	4.694 ± 0.393	106.22 ± 1.15
	4(N=5)	2.672 ± 0.119	1.836 ± 0.250	144.42 ± 0.94	4.488 ± 0.330	103.66 ± 1.11
	46(N=5)	2.582 ± 0.162	2.020 ± 0.213	147.80 ± 1.27	4.408 ± 0.298	103.90 ± 1.42
	71(N=2)	2.670 ± 0.141	1.710 ± 0.000	147.05 ± 0.07	4.940 ± 0.438	102.40 ± 2.12
Group 1(Female)	-2(N=5)	2.780 ± 0.091	1.734 ± 0.363	143.64 ± 3.40	5.002 ± 0.360	106.30 ± 0.94
	4(N=5)	2.692 ± 0.039	1.730 ± 0.279	144.58 ± 2.20	4.692 ± 0.106	105.50 ± 1.19
	46(N=5)	2.654 ± 0.105	1.504 ± 0.201	146.70 ± 1.71	4.506 ± 0.097	105.04 ± 1.13
	71(N=2)	2.680 ± 0.042	1.660 ± 0.509	146.40 ± 2.12	4.100 ± 0.156	104.85 ± 0.92
Group 2(Male)	-2(N=5)	2.696 ± 0.139	1.560 ± 0.200	145.98 ± 0.93	4.910 ± 0.228	105.34 ± 0.74
	4(N=5)	2.698 ± 0.151	1.708 ± 0.113	144.36 ± 0.85	4.656 ± 0.423	103.44 ± 1.07
	46(N=5)	2.676 ± 0.134	1.726 ± 0.423	147.62 ± 0.43	4.718 ± 0.255	103.04 ± 1.96
	71(N=2)	2.725 ± 0.049	1.705 ± 0.375	146.25 ± 0.92	4.935 ± 0.120	102.90 ± 2.12

Group 2(Female)	-2(N=5)	2.780 ± 0.172	1.200 ± 0.260	143.76 ± 1.43	4.760 ± 0.406	105.74 ± 1.68
	4(N=5)	2.694 ± 0.105	1.580 ± 0.174	142.96 ± 1.08	4.460 ± 0.126	104.46 ± 2.01
	46(N=5)	2.558 ± 0.033	1.440 ± 0.220	145.98 ± 1.45	4.540 ± 0.313	104,76 ± 2.28
	71(N=2)	2.615 ± 0.035	1.585 ± 0.078	145.60 ± 1.84	4.735 ± 0.375	104.10 ± 1.13
Group 3(Male)	-2(N=5)	2.696 ± 0.139	1.502 ± 0.345	144.62 ± 1.65	4.556 ± 0.308	106.26 ± 0.63
	4(N=5)	2.700 ± 0.098	1.804 ± 0.256	144.66 ± 1.32	4.484 ± 0.417	103.64 ± 1.13
	46(N=5)	2.632 ± 0.129	1.754 ± 0.401	147.90 ± 1.33	4.348 ± 0.249	104.52 ± 1.80
	71(N=2)	2.850 ± 0.028	1.360 ± 0.481	148.00 ± 1.56	4.435 ± 0.742	102.95 ± 1.63
Group 3(Female)	-2(N=5)	2.648 ± 0.105	1.324 ± 0.552	142.84 ± 0.53	4.564 ± 0.274	105.28 ± 2.30
	4(N=5)	2.630 ± 0.054	1.500 ± 0.278	142.96 ± 1,36	**4.252*** ± 0.365	104.18 ± 2.50
	46(N=5)	**2.446*** ± 0.126	1.306 ± 0.446	144.96 ± 1.41	4.588 ± 0.183	103.40 ± 1.08
	71(N=2)	2.510 ± 0.071	1.290 ± 0.184	144.50 ± 0.57	4.670 ± 0.396	102.80 ± 2.12

Note: “N” was cynomolgus monkey number of each group. * = $p < 0.05$.

Table S7. Abnormal mean value (pg/mL) and change range (%) of serum cytokines in cynomolgus monkey during the experiment.

Index	Day(s) Relative to Start Date	Male					Female				
		Group 1	Group 2		Group 3		Group 1	Group 2		Group 3	
		Mean	Mean	%Diff	Mean	%Diff	Mean	Mean	%Diff	Mean	%Diff
TNF-α	D-2	4.520	4.374	-3.2	4.460	-1.3	4.146	4.016	-3.1	4.510	8.8
	D1	4.618	4.338	-6.1	**4.018***	-13.0	3.524	4.096	16.2	4.288	21.7
	D2	4.418	4.880	10.5	4.512	2.1	5.138	**4.022***	-21.7	4.944	-3.8
	D43	4.572	4.326	-5.4	11.660	155.0	4.264	6.278	47.2	3.926	-7.9
	D44	3.902	3.548	-9.1	7.930	103.2	3.598	10.044	179.2	4.060	12.8
	D71	3.745	3.435	-8.3	5.540	45.5	3.025	4.255	40.7	4.125	36.4
IL-2	D-2	20.022	20.338	1.6	20.470	2.2	19.778	19.610	-0.8	19.948	0.9
	D1	19.508	20.208	3.6	19.632	0.6	18.400	19.294	4.9	19.272	4.7
	D2	19.956	19.506	-2.3	19.380	-2.9	19.116	18.788	-1.7	19.134	0.1
	D43	19.762	20.012	1.3	27.412	38.7	19.634	21.998	12.0	18.364	-6.5
	D44	17.712	**18.826***	6.3	**26.090***	47.3	18.468	24.384	32.0	18.424	-0.2
	D71	18.610	18.930	1.7	20.860	12.1	18.035	17.590	-2.5	18.315	1.6

IL-4	D-2	6.418	6.312	-1.7	6.096	-5.0	6.348	5.946	-6.3	6.368	0.3
	D1	6.558	6.418	-2.1	6.586	0.4	5.878	5.974	1.6	6.368	8.3
	D2	6.312	6.000	-4.9	5.988	-5.1	5.814	5.456	-6.2	6.504	11.9
	D43	6.482	6.336	-2.3	7.624	17.6	6.284	6.258	-0.4	**5.636***	-10.3
	D44	5.834	5.488	-5.9	6.600	13.1	5.892	6.180	4.9	5.472	-7.1
	D71	6.160	5.455	-11.4	5.830	-5.4	5.640	5.705	1.2	5.765	2.2
IL-5	D-2	0.672	0.488	-27.4	0.620	-7.7	0.515	0.460	-10.7	0.518	0.6
	D1	0.512	0.478	-6.6	0.500	-2.3	0.530	0.493	-7.1	0.524	-1.1
	D2	0.560	0.506	-9.6	0.486	-13.2	0.533	0.458	-14.1	0.514	-3.5
	D43	0.468	0.642	37.2	**2.124***	353.8	0.448	0.572	27.7	1.346	200.4
	D44	0.417	0.680	63.2	**2.078***	398.7	0.400	0.950	137.5	1.733	333.1
	D71	0.360	0.410	13.9	0.505	40.3	0.320	0.330	3.1	0.415	29.7
IL-6	D-2	6.502	9.354	43.9	7.134	9.7	8.212	6.280	-23.5	6.732	-18.0
	D1	9.658	22.360	131.5	30.792	218.8	9.202	14.966	62.6	20.648	124.4
	D2	9.236	31.970	246.1	23.560	155.1	10.222	12.646	23.7	19.290	88.7
	D43	7.906	10.626	34.4	**25.744***	225.6	10.618	11.976	12.8	16.554	55.9
	D44	6.948	8.538	22.9	12.320	77.3	8.336	12.506	50.0	9.826	17.9
	D71	6.225	4.750	-23.7	7.935	27.5	5.805	5.260	-9.4	6.090	4.9

Note: "*" indicates statistically significant difference compared with the same sex Group 1 (negative control group) ($P \leq 0.05$).
%Diif = (mean of all test groups - mean of negative control group)/mean of control group * 100.

Table S8. Antibody data in cynomolgus monkey

Sampling time	Group	Male		Female	
		The incidence of positive samples	Range of antibody titers	The incidence of positive samples	Range of antibody titers
D-2 (N=5)	Group 2	0/5	/	0/5	/
	Group 3	0/5	/	0/5	/
D14 (N=5)	Group 2	5/5	1:400-1:3200	5/5	1:400-1:3200
	Group 3	5/5	1:400-1:3200	5/5	1:1600-1:12800
D28 (N=5)	Group 2	5/5	1:12800-1:204800	5/5	1:12800-1:102400
	Group 3	5/5	1:51200-1:819200	5/5	1:102400-1:409600
D42 (N=5)	Group 2	5/5	1:12800-1:204800	5/5	1:12800-1:102400
	Group 3	5/5	1:204800-1:819200	5/5	1:102400-1:409600
D57 (N=2)	Group 2	2/2	1:12800-1:51200	2/2	1:51200-1:102400
	Group 3	2/2	1:204800	2/2	1:102400-1:204800
D71 (N=2)	Group 2	2/2	1:12800-1:51200	2/2	1:12800
	Group 3	2/2	1:102400	2/2	1:51200-1:102400

Note: “N” was cynomolgus monkey number of each group. “/” indicates the negative without titer range, and the incidence result is expressed as “number of animals with ADA confirmed antibody positive/number of animals participating in the determination”.

Table S9. Statistical data of organ weight

Group	Day(s) Relative to Start Date	Brain (g)	Thymus (g)	Heart (g)	The main bronchus of the lung (g)	Liver (g)	Spleen (g)	Kidney (g)	Adrenal gland (g)	Testis (g)	Epididymis (g)	Prostate (g)	Thyroid gland / parathyroid glands (g)
Group1 (Male)	46	71.672 ± 3.956	1.489 ± 0.658	13.275 ± 2.775	17.392 ± 3.618	59.414 ± 13.491	3.187 ± 0.794	13.81 ± 3.279	0.678 ± 0.105	10.968 ± 10.720	2.275 ± 2.071	1.086 ± 0.766	0.190 ± 0.023
	71	73.102 ± 5.582	3.500 ± 0.053	12.36 ± 1.579	15.706 ± 0.897	56.856 ± 3.685	2.662 ± 0.528	12.371 ± 2.052	0.502 ± 0.119	2.044 ± 0.156	1.012 ± 0.055	0.592 ± 0.001	0.228 ± 0.016
Group2 (Male)	46	68.923 ± 5.648	2.402 ± 0.720	10.969 ± 2.201	15.255 ± 1.848	62.101 ± 14.447	4.279 ± 1.282	13.212 ± 2.245	0.480 ± 0.068	6.094 ± 8.029	1.387 ± 1.053	0.605 ± 0.532	0.356 ± 0.139
	71	70.960 ± 2.584	2.573 ± 0.282	13.074 ± 1.649	18.087 ± 1.958	16.553 ± 11.166	5.685 ± 1.696	12.578 ± 0.860	0.450 ± 0.071	11.471 ± 13.099	2.323 ± 1.520	1.433 ± 1.027	0.219 ± 0.045
Group3 (Male)	46	70.880 ± 4，772	2.656 ± 0.916	13.939 ± 2。650	16.392 ± 2.024	58.806 ± 10.130	3.066 ± 0.401	12.180 ± 2.502	0.587 ± 0.069	11.292 ± 16.746	2.531 ± 2.820	1.113 ± 1.220	0.255 ± 0.041
	71	66.422 ± 2.378	2.841 ± 0.399	11.804 ± 0.954	17.551 ± 2.803	54.663 ± 7.316	3.370 ± 0.491	1.660 ± 2.160	0.442 ± 0.013	2.058 ± 0.773	0.720 ± 0.030	0.368 ± 0.210	0.216 ± 0.028

		Brain (g)	Thymus (g)	Heart (g)	The main bronchus of the lung (g)	Liver (g)	Spleen (g)	Kidney (g)	Adrenal gland (g)	Uterus (with cervix) (g)	Ovary (g)	Thyroid gland / parathyroid glands (g)
Group1 (Female)	46	64.285 ± 2.564	2.009 ± 0.452	8.933 ± 1.349	13.160 ± 1.555	49.615 ± 5.667	2.454 ± 0.565	10.287 ± 0.832	0.499 ± 0.034	6.221 ± 0.893	0.280 ± 0.044	0.218 ± 0.042
	71	66.318 ± 4.806	3.565 ± 1.344	10.294 ± 0.128	16.537 ± 0.873	53.892 ± 0.482	3.179 ± 1.314	10.873 ± 0.215	0.536 ± 0.035	6.114 ± 1.555	0.369 ± 0.006	0.282 ± 0.133
Group2 (Female)	46	63.912 ± 3.905	1.570 ± 0.765	10.089 ± 1.095	13.854 ± 2.269	50.105 ± 5.269	3.561 ± 1.170	11.307 ± 2.074	0.591 ± 0.138	8.045 ± 1.889	0.301 ± 0.061	0.281 ± 0.112
	71	67.444 ± 0.312	2.044 ± 0.551	11.115 ± 4.049	14.572 ± 4.364	56.636 ± 10.501	3.154 ± 0.612	11.571 ± 1.912	0.493 ± 0.092	7.034 ± 3.884	0.414 ± 0.083	0.274 ± 0.112
Group3 (Female)	46	62.737 ± 0.027	1.679 ± 0.696	9.515 ± 1.129	15.926 ± 3.187	48.458 ± 9.183	2.547 ± 0.163	10.002 ± 2.234	0.550 ± 0.157	8.389 ± 0.471	0.315 ± 0.117	0.273 ± 0.878
	71	57.601 ± 6.199	1.230 ± 0.807	10.074 ± 0.692	18.230 ± 1.652	54.120 ± 1.044	2.502 ± 0.484	11.556 ± 0.502	0.467 ± 0.089	6.094 ± 1.592	0.328 ± 0.78	0.204 ± 0.033

Table S10. Statistical data of visceral-body ratio

Group	Day(s) Relative to Start Date	Brain (%)	Thymus (%)	Heart (%)	The main bronchus of the lung (%)	Liver (%)	Spleen (%)	Kidney (%)	Adrenal gland (%)	Testis (%)	Epididymis (%)	Prostate (%)	Thyroid gland / parathyroid glands (%)
Group1 (Male)	46	1.888 ± 0.422	0.040 ± 0.025	0.340 ± 0.026	0.446 ± 0.038	1.517 ± 0.108	0.820 ± 0.014	0.356 ± 0.019	0.018 ± 0.002	0.243 ± 0.187	0.051 ± 0.035	0.025 ± 0.010	0.005 ± 0.001
	71	2.186 ± 0.190	0.105 ± 0.000	0.340 ± 0.051	0.469 ± 0.022	1.700 ± 0.128	0.080n ± 0.017	0.370 ± 0.065	0.015 ± 0.003	0.061 ± 0.004	0.030 ± 0.002	0.018 ± 0.000	0.007 ± 0.000
Group2 (male)	46	2.085 ± 0.457	0.071 ± 0.006	0.323 ± 0.024	0.456 ± 0.076	1.874 ± 0.590	0.125 ± 0.019	0.394 ± 0.076	0.014 ± 0.003	0.159 ± 0.192	0.038 ± 0.023	0.017 ± 0.012	0.011 ± 0.005
	71	1.916 ± 0.436	0.070 ± 0.021	0.348 ± 0.023	0.482 ± 0.041	1.736 ± 0.039	0.149n ± 0.016	0.336 ± 0.042	0.012 ± 0.000	0.275 ± 0.293	0.059 ± 0.029	0.036 ± 0.020	0.006 ± 0.000
Group3 (Male)	46	1.928 ± 0.384	0.077 ± 0.039	0.372 ± 0.051	0.442 ± 0.065	1.570 ± 0.156	0.085 ± 0.025	0.324 ± 0.030	0.016 ± 0.004	0.236 ± 0.322	0.057 ± 0.050	0.025 ± 0.022	0.007 ± 0.002
	71	1.986 ± 0.038	0.841 ± 0.002	0.315 ± 0.014	0.519 ± 0.021	1.618 ± 0.024	1.100n ± 0.003	0.344 ± 0.023	0.013 ± 0.002	0.060 ± 0.156	0.022 ± 0.004	0.011 ± 0.008	0.006 ± 0.000

		Brain (%)	Thymus (%)	Heart (%)	The main bronchus of the lung (%)	Liver (%)	Spleen (%)	Kidney (%)	Adrenal gland (%)	Uterus (With cervix) (%)	Ovary (%)	Thyroid gland / parathyroid glands (%)
Group1 (Female)	46	2.350 ± 0.162	0.073 ± 0.011	0.326 ± 0.044	0.479 ± 0.030	1.807 ± 0.114	0.089 ± 0.014	0.377 ± 0.045	0.018 ± 0.001	0.226 ± 0.018	0.010 ± 0.002	0.008 ± 0.002
	71	2.173 ± 0.448	0.113 ± 0.028	0.336 ± 0.052	0.537 ± 0.044	1.758 ± 0.253	0.101 ± 0.029	0.354 ± 0.041	0.018 ± 0.004	0.204 ± 0.078	0.012 ± 0.002	0.009 ± 0.003
Group2 (Female)	46	2.381 ± 0.257	0.057 ± 0.021	0.374 ± 0.022	0.510 ± 0.025	1.858 ± 0.123	0.129 ± 0.027	0.416 ± 0.031	0.022 ± 0.005	0.295 ± 0.047	0.011 ± 0.002	0.010 ± 0.004
	71	2.172 ± 0.371	0.064 ± 0.007	0.347 ± 0.071	0.457 ± 0.063	1.795 ± 0.035	0.090 ± 0.004	0.420 ± 0.047	0.016 ± 0.000	0.216 ± 0.087	0.013 ± 0.000	0.009 ± 0.002
Group3 (Female)	46	2.203 ± 0.135	0.061 ± 0.029	0.333 ± 0.029	0.557 ± 0.098	1.685 ± 0.129	1.090 ± 0.015	0.347 ± 0.038	0.019 ± 0.003	0.295 ± 0.030	0.001 ± 0.004	0.010 ± 0.004
	71	2.084 ± 0.100	0.045 ± 0.022	0.366 ± 0.032	0.669 ± 0.164	1,977 ± 0.345	0.090 ± 0.004	0.420 ± 0.047	0.017 ± 0.001	0.218 ± 0.024	0.012 ± 0.001	0.007 ± 0.000

Table S11. Statistical data of visceral-brain ratio

Group	Day(s) Relative to Start Date	Thymus (%)	Heart (%)	The main bronchus of the lung (%)	Liver (%)	Spleen (%)	Kidney (%)	Adrenal gland (%)	Testis (%)	Epididymis (%)	Prostate (%)	Thyroid gland / parathyroid glands (%)
Group1 (Male)	46	2.068 ± 0.902	18.451 ± 3.047	24.187 ± 4.095	82.5773 ± 15.397	4.415 ± 0.857	19.4071 ± 3.597	0.944 ± 0.115	14.950 ± 13.948	3.108 ± 2.688	1.487 ± 0.971	0.265 ± 0.023
	71	4.805 ± 0.440	16.882 ± 0.871	21.595 ± 2.896	77.810 ± 0.901	3.624 ± 0.446	16.865 ± 1.519	0.695 ± 0.216	2.806 ± 0.428	1.386 ± 0.030	0.812 ± 0.063	0.312 ± 0.002
Group2 (male)	46	3.525 ± 1.235	16.165 ± 4.616	22.337 ± 4.086	90.717 ± 22.680	6.340 ± 2.437	19.388 ± 4.441	0.702 ± 0.128	9.485 ± 12.908	2.098 ± 1.758	0.920 ± 0.878	0.510* ± 0.159
	71	3.622 ± 0.268	18.481 ± 2.988	25.559 ± 3.678	92.735 ± 19.069	8.062 ± 2.680	17.761 ± 1.850	0.636 ± 0.122	16.511 ± 19.056	3.315 ± 2.261	2.046 ± 1.522	0.311 ± 0.074
Group3 (Male)	46	3.815 ± 1.519	19.559 ± 2.381	23.112 ± 2.127	82.639 ± 9.090	4.356 ± 0.817	17.085 ± 2.354	0.833 ± 0.140	15.034 ± 21.911	3.429 ± 3.637	1.515 ± 1.578	0.359 ± 0.049
	71	4.291 ± 0.754	17.809 ± 2.074	26.516 ± 5.169	82.546 ± 13.969	5.090 ± 0.921	17.623 ± 3.883	0.666 ± 0.005	3.120 ± 1.275	1.085 ± 0.007	0.550 ± 0.297	0.325 ± 0.053

		Thymus (%)	Heart (%)	The main bronchus of the lung (%)	Liver (%)	Spleen (%)	Kidney (%)	Adrenal gland (%)	Uterus (With cervix) (%)	ovary (%)	Thyroid gland / parathyroid glands (%)
Group1 (Female)	46	3.124 ± 0.702	13.947 ± 2.463	20.444 ± 1.917	77.299 ± 9.839	3.818 ± 0.889	16.050 ± 1.870	0.775 ± 0.034	9.663 ± 1.208	0.436 ± 0.068	0.342 ± 0.077
	71	5.463 ± 2.422	15.551 ± 0.799	25.049 ± 3.131	81.451 ± 5.177	4.878 ± 2.335	16.450 ± 1.516	0.808 ± 0.006	9.159 ± 1.681	0.558 ± 0.032	0.434 ± 0.232
Group2 (Female)	46	2.419 ± 1.026	15.764 ± 0.918	21.662 ± 3.182	78.285 ± 4.056	5.527 ± 1.534	17.683 ± 3.026	0.933 ± 0.261	12.612 ± 3.098	0.468 ± 0.065	0.434 ± 0.159
	71	3.032 ± 0.831	16.494 ± 6.080	21.621 ± 6.571	84.013 ± 15.959	4.678 ± 0.987	17.163 ± 2.914	0.731 ± 0.140	10.442 ± 5.807	0.614 ± 0.127	0.407 ± 0.169
Group3 (Female)	46	2.715 ± 1.213	15.162 ± 1.578	25.297 ± 4.306	76.916 ± 10.822	4.075 ± 0.448	15.862 ± 2.778	0.8712 ± 0.206	13.3924 ± 0.998	0.499 ± 0.180	0.440 ± 0.158
	71	2.186 ± 1.165	17.526 ± 0.685	31.953 ± 6.307	94.603 ± 11.994	4.324 ± 0.374	20.132 ± 1.295	0.807 ± 0.068	10.491 ± 1.634	0.566 ± 0.075	0.352 ± 0.020

Table S12. The main gross findings and associated histopathological changes of euthanized rats at necropsy

Group	Animal number	Sex	Main findings at necropsy	Histopathological changes
Group 5 (Test sample) (SARS-CoV-2 Inactivated Vaccine (Vero cells, Delta strain), 20 ug/mL)	2120793	Male	White nodules (gastrocnemius muscle) were administered bilaterally, about 0.3cm×0.2cm×0.2cm in size	Granulomatous inflammation, mild
	2120797	Female	On the left side, the administration area (gastrocnemius muscle) was white nodules, about 0.2cm×0.2cm×0.2cm in size	Granulomatous inflammation, mild
	2120798	Female	Bilateral administration local (gastrocnemius muscle) white nodules, the size of the left side about 0.4cm×0.4cm×0.2cm, the size of the right side about 0.4cmx0.2cm×0.2cm	Granulomatous inflammation, moderate
	2120799	Female	Bilateral administration local (gastrocnemius muscle) white nodules, the left size about 0.4cm×0.3cm×0.2cm, the right size about 0.2cm×0.2cmx0.2cm	Granulomatous inflammation, moderate
	2120800	Female	The right administration area (gastrocnemius muscle) white nodules, about 0.3cm×0.2cm×0.2cm in size	Granulomatous inflammation, moderate

Table S13. Summary of injection local lesions in euthanized cynomolgus monkeys

Time	Group/Sex	Group 1 / Male	Group 2 / Male	Group 3 / Male	Group 1 / Female	Group 2 / Female	Group 3 / Female
3 days after the last dose (D46)	**Dose**	0	1	5	0	1	5
	Check the animal count	3	3	3	3	3	3
Injury of injection site	**Inujury degree**						
Granulomatous inflammation	Mild	0	1	1	0	2	1
	Moderate	0	0	2	0	0	0
	Severe	0	0	0	0	0	2
	Total	0	1	3	0	2	3
Inflammatory cell infiltration	Mild	0	2	0	1	0	0
	Total	0	2	0	1	0	0
Hemorrhage / muscle fiber necrosis	Mild	0	0	0	1	0	0
	Total	0	0	0	1	0	0
Time	**Group/Sex**	**Group 1 / Male**	**Group 2 / Male**	**Group 3 / Male**	**Group 1 / Female**	**Group 2 / Female**	**Group 3 / Female**
End of recovery period (D71)	**Dose**	0	1	5	0	1	5
	Check the animal count	2	2	2	2	2	2
Injury of injection site	**Inujury degree**						
Granulomatous inflammation	Mild	0	1	0	0	0	0
	Moderate	0	1	0	0	2	1
	Severe	0	0	2	0	0	1
	Total	0	2	2	0	2	2