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Development of SARS-Cov-2 Circulating Immune Complex Candidate, (CRCx) as A New Promising Vaccine Eliciting Broad Immune Response.

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Abstract

There is a need to develop a universal vaccine that can boost immunity to coronaviruses if some modifications in their structure occur. This is what we are dealing with nowadays: a virus that can mutate its structure, while immunity is standing still in facing the virus. We report on preclinical trials of CRCx 3 and CRCx 2 vaccine candidates in inducing an elevated level of positive neutralizing antibodies as well as a cellular immune response in an animal model to provide protection against SARS-CoV-2. Highly efficient protection against SARS-CoV-2 was obtained with three-dose immunization using 0.25 ml of CRCx vaccine with a 25-mm needle at 7-day intervals between successive injections. In addition, CRCx vaccine candidates exhibit efficient productivity and good genetic stability for vaccine manufacture. These results support the further evaluation of CRCx in a clinical trial.

Keywords: SARS-CoV-2, Vaccine, CD8+ Cytotoxic T Lymphocyte, CD4+ Helper Cells, Neutralization Antibodies, Spike (S) Glycoprotein, Immune Complex

Introduction

Severe acute respiratory syndrome coronavirus (SARS-CoV) emerged in 2003, and Middle East Respiratory Syndrome Coronavirus (MERS-CoV) in late 2012 [1,2]. COVID-19 is caused by a new positive-strand RNA coronavirus, SARS-CoV-2, which belongs to the Coronaviridae family. This family also includes the severe acute respiratory syndrome (SARS) coronavirus and the Middle East respiratory syndrome (MERS) coronavirus [3, 4]. Their genomes encode several major structural proteins and nonstructural proteins including membrane (E), spike (S), envelope (M), and nucleocapsid (N) proteins, approximately sixteen nonstructural proteins (nsp1–16), and five to eight accessory proteins [5]. Among them, the S protein plays an essential role in viral attachment, fusion, entry, and transmission. The S protein is the common target antigen for antibodies and vaccine development. After SARS-CoV-2 infection, distinct categories of antibodies circulate in serum, such as immunoglobulin G (IgG), immunoglobulin M (IgM), and immunoglobulin A (IgA). Two viral proteins are targeted: the S protein and the nucleoprotein (NP). S protein is abundant and highly expressed, but due to its biological function, it is unlikely that antibodies to NP have neutralizing activity.

Most of the recent vaccines for COVID-19 that employ injection of viral antigens or viral gene sequences are aimed at inducing neutralizing antibodies against the viral spike protein (S), preventing uptake through the human ACE2 receptor, thus blocking infection [6,7]. Neutralizing antibodies elicited by prior infection or vaccination are likely to be key for future protection of individuals and populations against SARS-CoV-2. Moreover, passively administered antibodies are among the most promising therapeutic and prophylactic anti-SARS-CoV-2 agents. However, the degree to which SARS-CoV-2 will adapt to evade neutralizing antibodies is unclear.

Previous reports have provided evidence that neutralizing antibodies are potent enough to prevent viral infection. Furthermore, they strongly suggest that neutralizing antibody-based vaccines could provide effective protection against coronavirus, and the antibodies could constitute a promising cornerstone for the efficacy of an effective vaccine against any viral infection. However, we have concluded that neutralizing antibodies could carry a pathogenic effect that counters its protective one, and the coronavirus could use these nAbs to mask a proportion of corresponding antigens in immune-complex form (Ag/nAbs) antigen/neutralizing antibody for a long time, preventing its attack by CD8+ cytotoxic T cells. Based on our assumptions, we have discussed the possibility of developing a new in vitro vaccine comprising the peptide combination Ag/non-specific Abs as the extrinsic immune complex (ICA, ICB, and ICC) is completely dissimilar from the existing intrinsic circulating immune complex that comprises coronavirus antigen (M, N, and S and its specific neutralizing antibodies IC1, IC2, and IC3 as a circulating immune complex).

The CRCx vaccine candidate is an immune-peptide combination that was created to function as a novel therapeutic intervention for curing and preventing coronavirus infection. By coupling Ag/nonspecific Abs in one form, it differs from an existing intrinsic circulating immune complex (CIC) that shares Ag/specific Abs. This changes the self-tolerance, which refers to the lack of immune response to the intrinsic CIC because of central (thymic selection) or peripheral (lack of co-stimulation) tolerance. We found that if we injected an extrinsic noncomplex trigger, to some extent as the same intrinsic CIC, it could initiate specific immune recognition against the tolerated CIC.

This novel immune peptide complex CRC**x** was designed to stimulate the attention of CD8+ T-cells towards the intruder's extrinsic CIC and the intrinsic CIC to put the CD8+ T-cells in a state of confusion to distinguish between the existing CIC and the similar intruder noncomplex CIC. These interactions can trigger a series of immunoregulatory responses involving both the innate and adaptive immune systems and including cross-presentation of antigens, activation of CD8 + T-cells and CD4 + T-cells, phagocytosis, complement-mediated antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC). Finally, the cytotoxic cells use both mechanisms to destroy viruses.

This type of vaccine can achieve a longer-lasting immune response and boostable memory response against coronavirus, as shown in Figures 1 and 2. Here, we report a study of CRCx vaccine candidates. The results show that its potency and safety in preclinical studies warrant further clinical evaluation.

Methods

Vaccine design and production

We isolated SARS-CoV-2 antigen and its specific neutralizing antibodies to develop preclinical in vitro neutralization and challenge models for inactivated SARS-CoV-2 vaccine candidates. Two formulated vaccine candidates CRCx were used in this study (Figure 1,2).

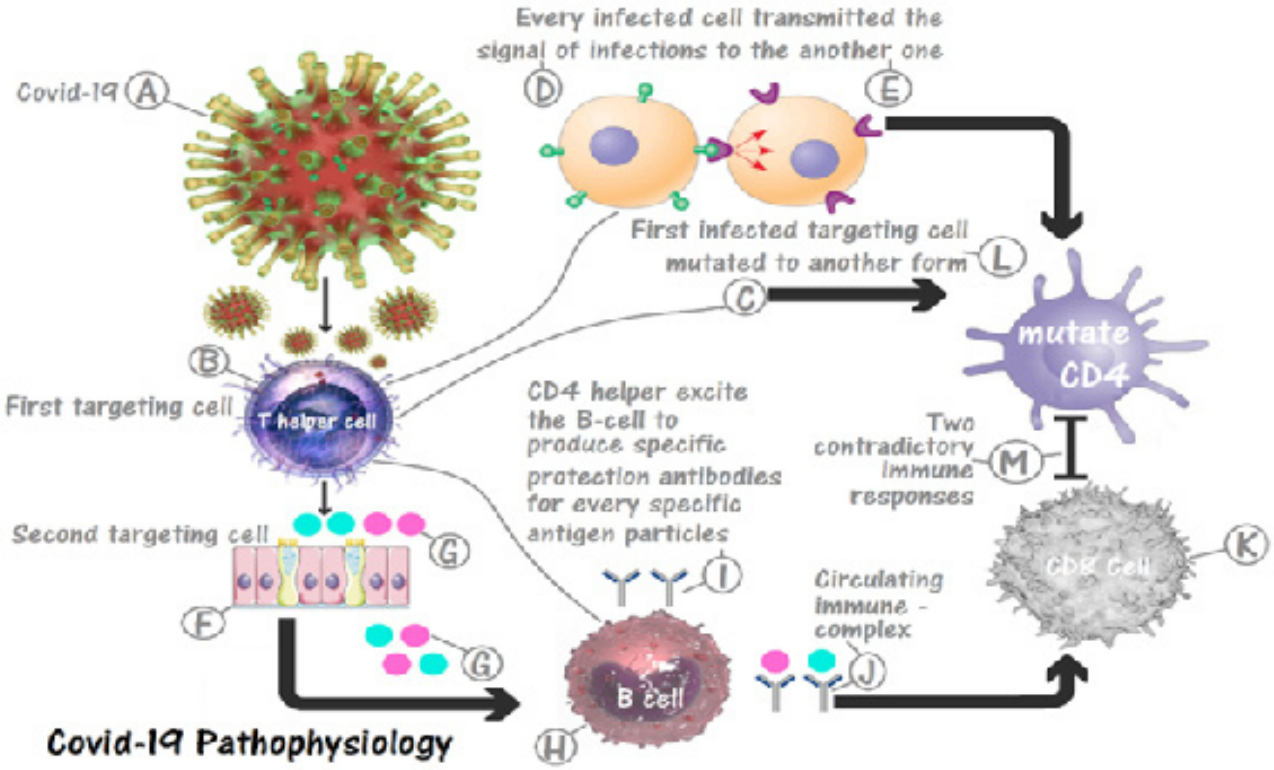


Figure1: Hypothetical mode of action for Coronavirus. (A) Virus particles, (B) CD4 T-cell as first susceptible cell, (C) evolution of the CD4+ T-cell mutating to be an unaccountable CD8+ T-cell, (D) tendencies of the first susceptible infected cell to infect other healthy ones and to be a new form of CD8+ T-cells, (E) newly formed infected first susceptible cell, (F) cells of the upper and lower respiratory tract as second susceptible cells, (G) productions of viral antigen particles from the second susceptible cells under the induction of first susceptible cells' stimulus of transmit ions signals, (H) CD4+ T-cell stimulating B-cells to produce negative neutralized coating antibodies to form a complex with antigen particles, (I) B-cells producing negative neutralizing antibodies, (J) formations of circulating immune complex comprising coronavirus antigens and its specific negative neutralizing antibodies, (K) CD8 + T-cells, (L) inhibitors effect of these complexes in preventing the cytotoxic CD8+ T-cells from attacking these viral particles, (M) the interaction mechanisms that originate as a result of antagonism between the newly formed mutated CD4 + T-cells and the normal CD8+ T-cells.

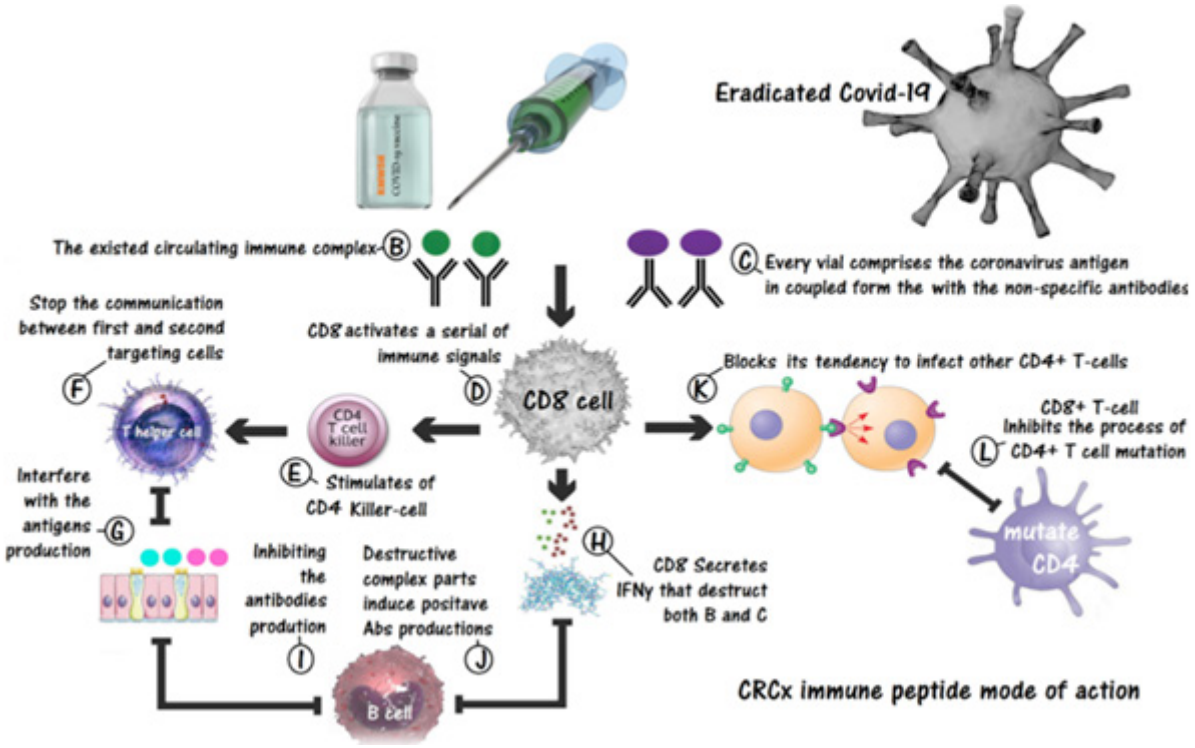


Figure 2: Immune stimulating action of the CRCx3 vaccine. (A) CRCx immune peptide, (B) Every vial comprises the coronavirus antigen in coupled form with non-specific antibodies, (C) existing circulating immune complex, (D) CD8+T-cell stimulated to induce a process of scanning and comparison between these non-complex combinations and those already existing during the vaccine injection. Also, CD8 stimulates a series of immune signals. (E) Stimulation of CD4+ killer cell, (F) stimulation of CD4+ Helper cell, (G) CD4+ T-cell sending a signal that inhibits the formations of the coronavirus antigens, (H) cytotoxic T-cells secreting IFNy that destroy the circulating immune complex and the intruder non-complex one, (J) destructive complex particles inducing positive antibody productions from B-cells. (K) Blocking of the tendency of CD4 to infect another cell and (L) CD8 + T-cells inhibiting the process of CD4+ T-cell mutation.

Vaccine A Composition: CRCx3

Vaccine A was formulated as three single-dose vials containing 0.75 ml of injectable solution. The first dose of the CRCx vaccine contained twenty-five µg of SARS-CoV-2 spike protein (S1 subunit), forty µg of anti-Nucleocapsid antibodies, and Fc of IgG mouse anti-Human IgM as an adjuvant immunogen dissolved in human albumin, phosphate buffer, and sodium chloride. The second dose of CRCx vaccine contained 25 µg of nucleocapsid antigen, 40 µg of anti-membrane antibodies dissolved in human albumin, and Fc of IgG mouse anti-Human IgM as an adjuvant immunogen dissolved in human albumin, phosphate buffer, and sodium chloride. The third dose of the CRCx vaccine contained twenty-five µg of membrane antigen, 40 µg of anti-spike (S1 subunit) antibodies, and Fc of IgG mouse anti-Human IgM as an adjuvant immunogen dissolved in human albumin, phosphate buffer, and sodium chloride. These were made ready for immunizing animal model groups with these non-immune complexes to induce the production of positive antibodies. The vaccine does not contain any stabilizers or preservatives.

Vaccine B Composition: CRCx2

Vaccine B was formulated as two single-dose vials containing 0.75 ml of injectable solution. The first dose of the CRCx vaccine (B) contains 25 µg of spike antigen (S1, S2 subunits), 40 µg of anti-spike antibodies (S1, S2 subunits), and Fc of IgG mouse anti-Human IgM as an adjuvant immunogen dissolved in human albumin, phosphate buffer, and sodium chloride. The second dose of CRCx vaccine (B) contains 25 µg of nucleocapsid antigen and 40 µg of anti-nucleocapsid antibodie Fc of IgG mouse anti-Human IgM as an adjuvant immunogen dissolved in human albumin, phosphate buffer, and sodium chloride.

Animal Study

60 female and male BALB/c mice at six to eight weeks old were housed in cages covered with barrier filters at biosafety level 3. All animals that participated in this research were in good health and were not involved in the other experimental procedures. All animals were allowed free access to water and diet and provided with 12-h light/dark cycle (temperature: 18-28°C, humidity: 40–70%). The mice were bred and maintained in a specific pathogen-free environment at the Laboratory Animal Center. They were divided into 12 groups with five mice each. The first six groups were designed to study the safety and efficacy of the vaccine candidates A and B as preventive vaccines. Group D was a placebo group, while the last six groups (E, F, G, and H) were designed to evaluate the safety and efficacy of the vaccines candidates as therapeutic vaccines.

Immunogenicity of CRCx as a prophylactic vaccine candidate.

The mice were injected with different immunization programs and various doses (0.25, 0.35, and 0.40 ml/dose) of vaccines A and B. We studied the immunogenicity of vaccine A (CRCx3). In the vaccine A trial, mice were intraperitoneally administered as high (0.40 ml/dose), middle (0.35 ml/dose), or low (0.25 ml/dose) dose of CRCx at day 0, and the levels of neutralization antibody (NAb) were evaluated at 7, 14, 21, and 28 days after injection. morever, mice were intraperitoneally administered a high (0.40 ml/dose) and low (0.25 ml/dose) dose of CRCx3 at day 0, a second dose was given at day 7, and the levels of neutralization antibody (NAb) were evaluated at 7, 14, 21, and 28 days after injection. Mouse groups were also intraperitoneally administered a high (0.40 ml/dose) and low (0.25 ml/dose) dose of CRCx3 at day 0, day 7, and day 14, and the levels of neutralization antibody (NAb) were evaluated at 7, 14, 21, and 28 days after injection (Figure 3).

We additionally studied the immunogenicity of vaccine B (CRCx2). Mice were intraperitoneally administered a high (0.40 ml/dose), middle (0.35 ml/dose), or low (0.25 ml/dose) dose of CRCx at day 0, and the levels of neutralization antibody (NAb) were evaluated at 7, 14, 21, and 28 days after injection. Also, mice were intraperitoneally administered a high (0.40 ml/dose) and low

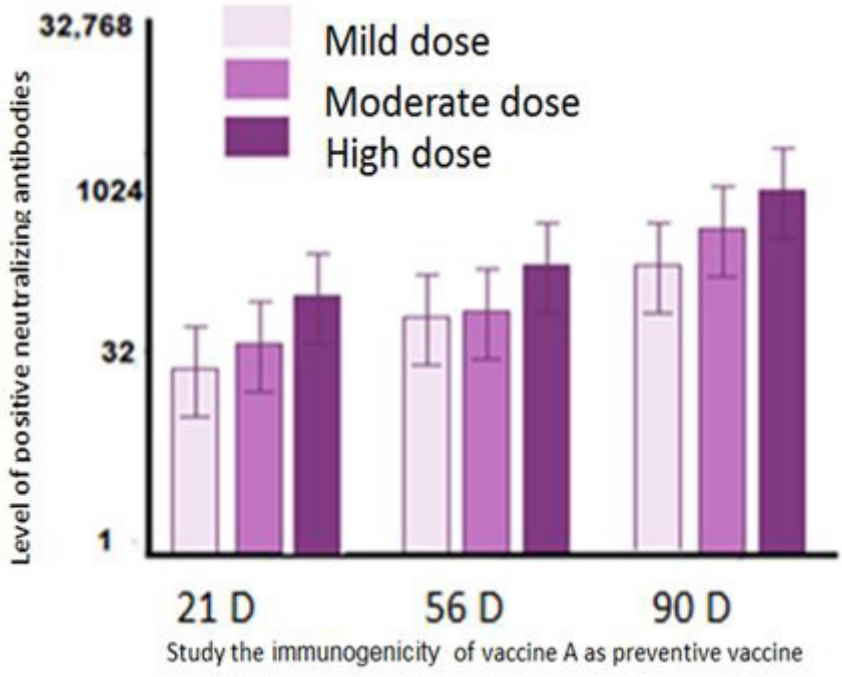


Figure 3: CRCx3 (vaccine a) immunization elicits a neutralizing antibody response in mouse model with different doses and immunization programs. Mouse neutralization antibody (NAb) levels with one-dose shot (D0) immunization of CRCx3 vaccine candidate A. Mice were injected intraperitoneally or intramuscular with a high (0.40 ml/dose), middle (0.35/dose), or low (0.25/dose) does at day 0, and the NAb levels at 7 days, 14 days, 21 days, and 28 days after the first immunization were tested by the microtitration method (n = 10). Mouse neutralization antibody (NAb) levels with two-dose shots immunization of CRCx3 vaccine

candidate (A). Mice were injected intraperitoneally or intramuscular with a high (0.40 ml/dose), middle (0.35/dose), or low (0.25/dose) at day 0 and day 7, and the NAb levels were evaluated at 7 days, 14 days, 21 days, and 28 days after the first immunization by the microtitration method (n = 10). Mouse neutralization antibody (NAb) levels with three-dose shots immunization of CRCx3 vaccine candidate A. Mice were injected intraperitoneally or intramuscularly with a high (0.40 ml/dose), middle (0.35/dose), or low (0.25/dose) does at day 0, day 7, and day 14, and the NAb levels were tested at 7 days, 14 days, 21 days, and 28 days after the first immunization by the microtitration method (n = 10).

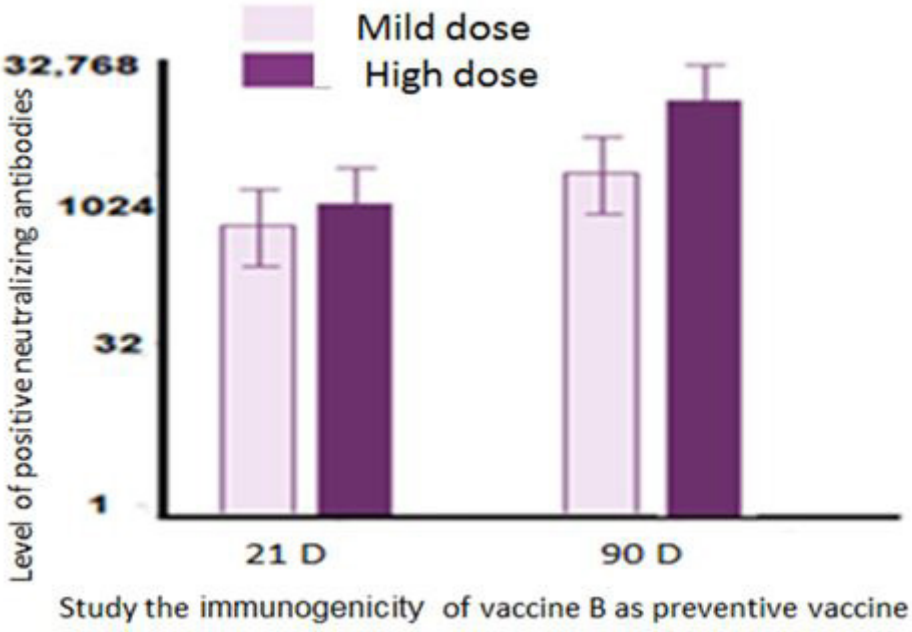


Figure 4: Immunization of CRCx2 (vaccine B) elicits a neutralizing antibody response in mouse model with different doses and immunization programs. Groups E and F were evaluated by the microtitration method (n = 10). The level of NAb was evaluated at days 21, 28, 56, and 90. Group E showed marked progress in eliciting Nab with a high dose with a higher titer than that of NAb in group F, which was immunized with a mild dose.

(0.25 ml/dose) dose of CRCx3 at day 0 with a second dose at day 7, and the levels of neutralization antibody (NAb) were evaluated at 7, 14, 21, and 28 days after injection (Figures 3 and 4).

Results

The results of vaccine A trial in a transgenic mouse model showed that the seroconversion rate in the high and middle-dose groups reached 100% at 21 days after immunization and 60% when using a low dose, and the immunization effect was time dependent. The NAb levels at 7, 14, and 21 days in the moderate and high-dose groups showed significant variation, and a significant variation was also observed between 21 and 28 days. In the high-dose group, a significant variation was observed only between 14 and 21 days.

We tested different immunization ptrograms (day 0, day 7, and day 14 at 7-day intervals. The seropositivity of the high, medium, and low-dose group for all three immunization programs reached 100% at 21 days after the third immunization and 76% after the second immunization, and 45% after the first immunization. The immunogenicity of the three-dose immunization program was significantly higher than that of the one-dose and two-dose immunization programs. The results showed that the three-dose (day 0/day 7/day 14) immunization program resulted in higher NAb levels than the one and two-dose programs in all groups at days 28 days.

We also analyzed the NAb levels in mice with high, middle, and low doses of the vaccine following one-dose (day 0), two-dose (day 0/day 21), and three-dose (day 0/day 7/day 14) immunization programs. We then checked the NAb levels at 28 days after the

first immunization to maintain the same starting and ending points. The results showed that the immunogenicity of the three-dose (day 0/day 7/day 14) immunization program was higher than that of both the one- and two-dose programs.

The results of vaccine B trials in the mouse model showed that the seroconversion rate in the high-dose group reached 100% at 14 days after immunization and 70% when using a low dose and the immunization effect was time dependent. The NAb levels at 7, 14, and 21 days in the high-dose groups showed no significant variation, but a significant variation between 21 and 28 day was observed.

We tested different immunization programs (day 0, day 7) at 7-day intervals at which two immunizations were applied. The seropositivity of the high and low-dose groups for both immunization programs reached 100% at 21 days after the second immunization and 55% after the first immunization. The seroconversion rate in the high and low-dose groups reached 50% at 7 days after immunization, and the immunization effect was time dependent.

The NAb levels at 7, 14, and 21 days in the low-dose group showed no significant variation, whereas a significant variation was observed between 21 and 28 days. In the high-dose group, a significant variation was observed only between 7 and 14 days. At day 24 (10 days after the third immunization using vaccine A (first sex groups) and the second immunization using vaccine B (second sex group)), all group of mice were intratracheally challenged with l06 TCID50 of SARS-CoV-2. Body temperatures of both the vaccinated group and placebo group fluctuated within the normal range after virus challenge from 0 to 7 days post-inoculation (dpi).

Moreover, blood was collected, and serum biochemical parameters were monitored at different time points after vaccination and challenge with the living virus. The protective efficacy of CRCx3 and CRCx2 against SARS-CoV-2 challenge at 14 days after the third immunization was evaluated. Changes in clinical signs (temperature, °C) were recorded. Viral loads were determined by real-time PCR in throat swabs (D) and anal swabs (E) obtained from mice at 3-, 5-, and 7-days post-inoculation. All placebo mice maintained a high viral load during the whole evaluation period after virus challenge in both throat and anal swabs. In contrast, the viral load in the throat swabs of the low-dose group peaked at 6.10 log10 copies/mL at 5 dpi and then decreased to 1.05 log10copies at 7 dpi, which was significantly lower than that of the placebo group.

Among the five mice in the low-dose group, three showed a nondeductible viral load at 7 dpi. The throat swabs of all five mice in the high-dose group were negative for viral load. Moreover, no viral load was detected in the anal swabs of two out of five mice in the high-dose group. At 7 dpi, all animals were euthanized to determine the viral load in the lung tissue and for pathological examination. No mice in the low-dose and high-dose groups had a detectable viral load in any lung lobe, which was significantly different from the results in the placebo group. In the placebo group, a high viral load was detected in the left lower lung, right lower lung, and right accessory lung, and the pathological histology analysis results showed severe interstitial pneumonia. Notably, only 3 of 7 sections of the lung lobes showed infection in the placebo group, possibly because the virus infection in the lung lobes dynamically changes.

Furthermore, all mice that received vaccination showed the normal lungs with mild, focused histopathological changes in a few lobes, demonstrating that CRCx vaccination could efficiently block the infection by SARS-CoV-2 and COVID-19 in mice. At 7 dpi, the mice treated with placebo produced low-level NAb with a titer of 1:16, whereas the NAb levels of the vaccinated mice were highest at 1:2,048 (average 1:860) in the high-dose group and 1:1,024 in the low-dose group (average 1:512). Taken together, all these results demonstrated that both low-dose and high-dose CRCx conferred highly efficient protection against SARS-CoV-2 in mice.

Immunogenicity of CRCx3 and CRCx2 as Therapeutic Vaccine Candidates

All mouse groups (G–L) were intratracheally challenged with l06 TCID 50 of SARS-CoV-2. Body temperatures of all groups and the placebo group were measured after virus challenge from 0 to 7 dpi. Blood was collected, and serum biochemical parameters

and immunological markers were monitored at different time points (Human anti CD40, Anti-CD8, CMV IgG, PAI1 Human Plasminogen Activator Inhibitor 1 (PAI1), CD4 T-cell count, CD8 T-cell count, D-Dimer, Inflammatory array (quantitative) IL-1 alpha, IL-1 beta, IL-4, IL-6, IL-8, IL-10, IL-13, MCP-1, IFN-gamma, TNF alpha, C-Reactive Protein, LDH Assay Kit/Cardiac Troponin I enzyme, Lactate Dehydrogenase, Serological detection for COVID-19 IgG, IgM in serum samples, and nasotracheal swab for detection of qualitative SARS-CoV-2 RNA).

At day 7 after virus inoculation and at day 28 after immunization with CRCx2 and CRCx3, groups G, H, and I were immunized intraperitoneally and intramuscularly with CRCx3 at three doses (0.25, 0.35, 0.40 ml/dose) using a syringe with a 25-mm needle with a high (0.40 ml/dose), middle (0.35 ml/dose), or low (0.25 ml/dose) dose at day 7. The second dose was given at day fourteen, and the last dose was given at day 21 (7-day interval). Groups J and K were immunized intraperitoneally and intramuscularly with CRCx2 with two doses (0.25, 0.40 ml/dose) using a syringe with a 25-mm needle with a high (0.40 ml/dose) and low (0.25 ml/dose) dose of at day 7, and the second dose was given at day 14 (also a 7-day interval). Group L had a placebo.

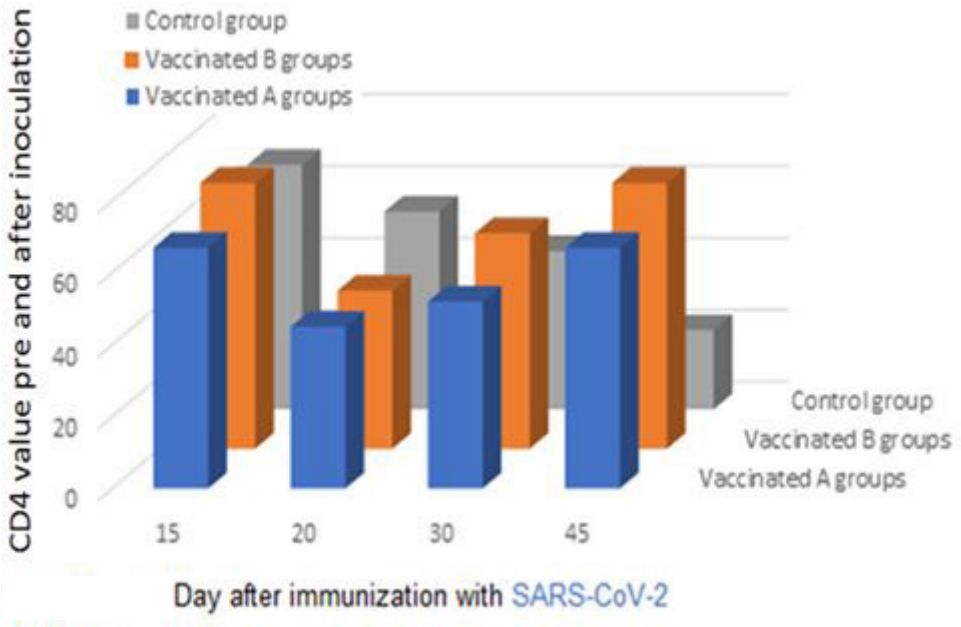
All mice in group G- L showed weight loss, became lethargic, and developed ruffled fur, a hunched posture, and rapid breathing. They had signs of pneumonia and marked elevation in body temperature, and most of all animals showed nervous manifestations and a marked elevation in all biochemical and biological markers at the end of day 6 after challenge with l06 TCID 50 of SARS-CoV-2. The protective efficacy of CRCx3 and CRCx 2 against SARS-CoV-2 challenge was evaluated at 14 days after the second immunization. In groups G, H, and I using CRCx3, about 80% of viral signs and symptoms disappeared at day 14 with low and moderate doses and later reached 100%.

Viral loads in throat and anal swabs obtained from mice at day 14 were decreased from 4.87 log10 copies/mL at day 7 to 0.5 log10 copies, which was significantly lower than that of the placebo group (6.4 log10 copies/mL) at day 7. At the end of day 28, no viral load was detected, and all biochemical and biological markers dramatically decreased to above normal values. The high-dose group showed complete recovery from all signs and symptoms, the viral load from real-time PCR was 0 log10 copies/mL, and there was marked adjustment of all biochemical and immunological markers from day 21 to day 28.

Using CRCx2 in groups I and J against SARS-CoV-2 challenge, at 14 days after the second immunization, a significant positive result was detected directly from day 7. All symptoms and signs completely disappeared with high or low doses, and no viral load was recorded at days 14, 21, and 28. All biochemical and immunological markers dramatically decreased to normal values in the high-dose group from day 21 to 28, while the low-dose group showed elevations in some immunological and biological markers at day 28. The results of immunological markers are shown in (Figures 5, 6, and 7).

We first performed a single intramuscular injection experiment in rats to evaluate the acute toxicity of RCx. In this study, 20 rats were divided into two groups (n = 10, 5 of each gender) and intramuscularly injected them with 2 doses (0.6, 0.8, 1.20 ml/dose) of CRCx or physiological saline as the control. After inoculation, all rats were continuously observed for 14 days and euthanized at day fifteen to assess systemic anatomy and for general observation. No cases of death, impending death, or obvious clinical signs were observed in any of the four groups over 14 consecutive days after vaccine inoculation. Moreover, there was no significant difference in weight or feeding state between the experimental groups and control group. No histopathologic changes were observed after euthanasia. Notably, the maximum tolerated dose (MTD) used for a single intramuscular injection in rats was 1.2 ml/rat, which is equivalent to 6 times the dose in humans, indicating the potentially good safety of CRCx in humans.

Systemic anaphylaxis due to CRCx was subsequently evaluated by intramuscular and intravenous injections in guinea pigs. Twenty-four male guinea pigs were divided into 4 groups (6 per group): a negative control group (physiological saline), a positive control group (human blood albumin, 20 mg/sensitization, 40 mg/stimulation), a low-dose group (0.1x dose/sensitization, 0.2x dose/stimulation), and a high-dose group (1 x dose/sensitization, 2 x dose/stimulation). Sensitization was performed on day 1, day 3, and day 5. The first stimulation (intravenous excitation via the foot) was performed for 3 out of 9 guinea pigs from each group at day 19, and secondary stimulation of the remaining animals of each group (6/9) was performed at day 26.


Control group
Vaccinated B groups
Vaccinated A groups
CD4 value pre and after inoculation
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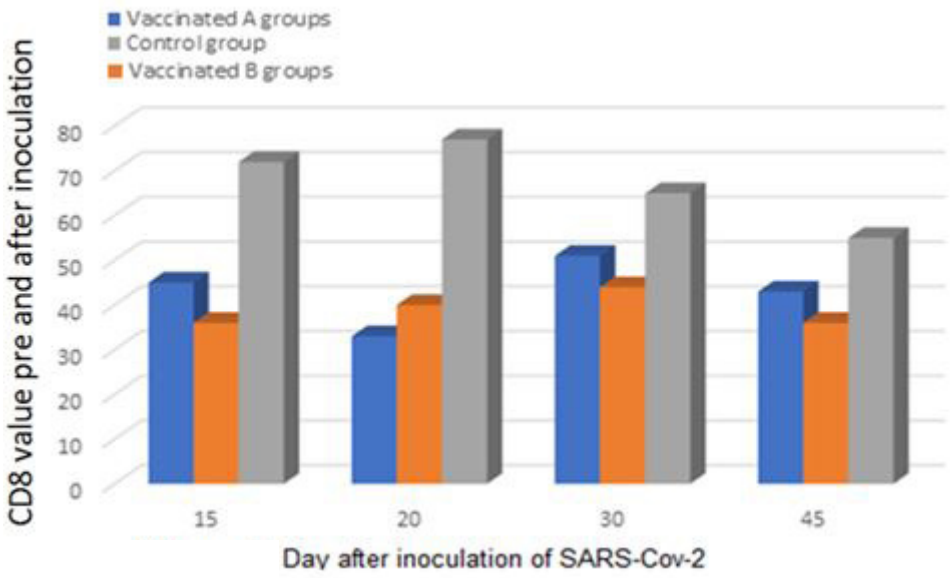
20
40
60
80
15
20
30
45
Day after immunization with SARS-CoV-2
Control group
Vaccinated B groups
Vaccinated A groups


Vaccinated A groups
Control group
Vaccinated B groups
CD8 value pre and after inoculation
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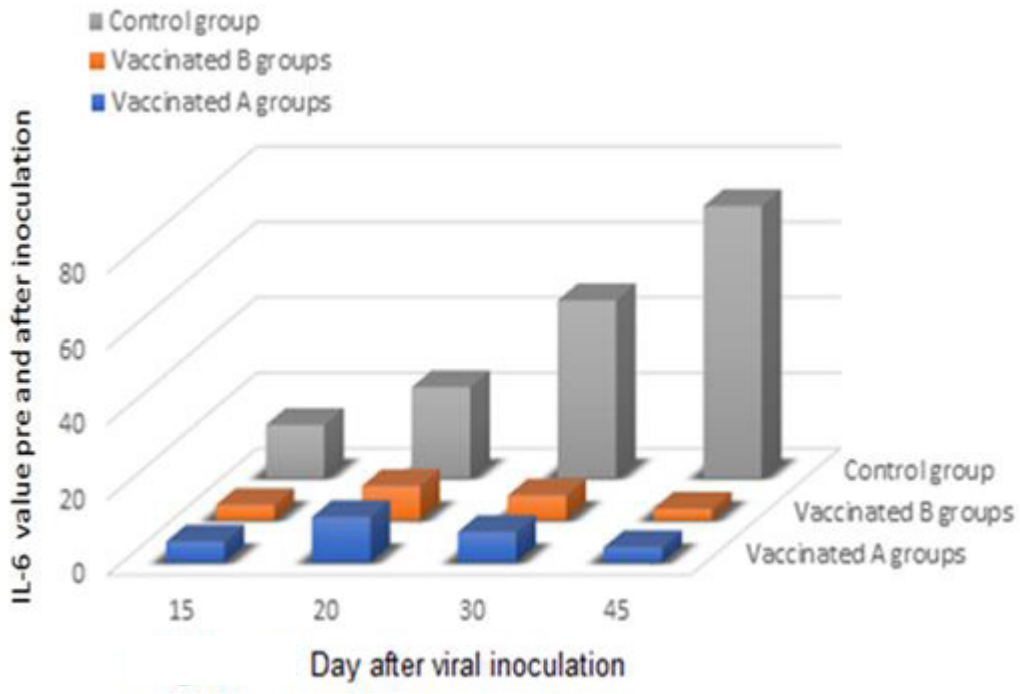
Day after inoculation of SARS-Cov-2



Figures 5,6,7: Immunological markers (CD4, CD8, and IL-6) before and after vaccination in groups A and B.

The results showed no abnormal reactions during the sensitization period by clinical observation and measurement of the body weight of the guinea pigs. No allergic reaction symptoms were found in the negative control group or experimental group on day 19 or day 26. The anaphylaxis result of the positive control group was highly positive (1/6 animals were positive, 3/6 animals were strongly positive, and 2/6 animals were extremely positive). In sharp contrast, in the low- and high-dose groups, no allergic reactions at day 19 and day 26 were found.

Discussion

The development of preventive and therapeutic vaccines with high immunogenicity and safety is crucial for control of the global COVID-19 pandemic and prevention of further illness and fatalities. Different types of conventional vaccines enter the race to obtain good results and safety, and one of them uses whole viruses to trigger an immune response [8, 9]. Subunit vaccines use pieces of the pathogen (often fragments of protein) to trigger an immune response [10, 11]. Nucleic acid vaccines use genetic material (either RNA or DNA) to provide cells with instructions to make antigens. In the case of COVID-19, this is usually the viral spike protein.

Once this genetic material goes into human cells, it uses our cells' protein factories to make antigens that will trigger an immune response. The advantages of such vaccines are that they are easy to make and cheap since the antigen is produced inside our own cells and in large quantities [12, 13]. viral vector vaccines also work by giving cells genetic instructions to produce antigens [14, 15]. However, it has been reported that the probabilities of reinfection after the use of diverse vaccines have not been resolved or prevented. moreover, more reports were recorded of the possibility of serious side effects after the first and second doses, like thrombosis with thrombocytopenia syndrome (TTS) after receiving the Johnson & Johnson/Janssen COVID-19 vaccine [16-18], as well as myocarditis or pericarditis among people ages 30 and younger who received a COVID-19 vaccine [19-20]. Most cases have been reported after mRNA COVID-19 vaccination (Pfizer BioNTech or Moderna), particularly in male adolescents and young adults in the United States from December, 14, 2020, through October 6, 2021. During this time, VAERS received 8, 638 reports of death (0.0021%) among people who received a COVID-19 vaccine [21-22]. Severe allergic reactions, including anaphylaxis, can also occur after any vaccination [23].

Most approved vaccines have traditionally focused on the induction of strong protective neutralizing antibodies against the target pathogen, thus aiming to confer immunity in vaccinated individuals in the long term to protect the body from the risk of infection

or recurrence. According to our postulation, the broadly neutralizing antibodies that are generated during vaccination exist in two forms. The first is a bound positive form where it effectively masks a proportion of the corresponding coronavirus antigen's structural and nonstructural protein in a complex form that prevents its elimination, controlling its activitie. The second is a free negative form, which is nonfunctional and non-neutralizing. This immune complex formation can explain the persistence of the viral infection.

Previous reports noted the importance of the immune complex as inflammatory mediator stimuli. An immune complex rises when the body's immune system generates antibodies against antigenic determinants of the host or foreign substances that recognize and bind to the antigen molecules. An immune complex is formed from a neutralizing Ag/Abs complex [24, 25]. Normally, the insoluble immune complexes that are formed are cleared by the phagocytic cells of the immune system, but when an excess of antigens/antibodies is present, the immune complexes are often deposited in tissues, where they can elicit complement activation and localized inflammation, resulting in the generation of tissue lesions in a variety of autoimmune diseases [26-28], which exacerbates the disease pathology.

We believe that our cells accumulate cumulative compounds or complexes inside or outside them, and these accumulations may increase to the point where the possibility of cellular asphyxiation may exist, depending on the level of accumulation, like when the endothelium of arteries and veins narrows, leading to blood clots. This leads us to ask ourselves, why do our cells produce these immune complexes, and how are these complexes the main cause by which the coronavirus can target us? Seemingly, our cells are in constant need for defense, so they produce many emergency antibodies that become unconsciously excited due to the repetition of multiple stimuli. Thus, immune cells initiate the production of these emergency negative antibodies to couple the stimuli to mitigate their impact, but they do not produce these immune complexes to remove the stimulus. Unfortunately, they create a bond, which leads to a chronic state, and the more the body produces these immune complexes, the greater the severity of any invader will become. We all have different levels of these emergency antibodies, which make us a possible target for any stimulus that might be viral. It is important to prevent the formation of these irrational forms of complexes to prevent viral infections.

Conclusion

We are trying to understand the behavior and the way in which the neutralizing antibodies works, as well as the reasons behind the ability of our humoral immune cells to produce these positive or negative neutralizing antibodies. We believe that the difference between antibodies for the same antigen may be the cornerstone for producing an effective vaccine and may also be the reason behind the failure of many vaccines in inducing long-term immunity. Here, we reported the development of SARS-CoV-2 immune complex vaccine candidates that can induce high levels of positive neutralizing antibodies and cellular immunity in animal models. CRCx is an immune peptide combination candidate that acts as a novel therapeutic and preventive vaccine for SARS-CoV-2 infection.

Both CRCx2 and CRCx3 can induce a highly efficient protection against SARS-CoV-2 without observable ADE or immunopathological exacerbations. The vaccine candidates were found to significantly reduce or nullify viral load and bronchoalveolar effects in animal models challenged with SARS-CoV-2 within 14 days after receiving the third dose of the vaccine candidate. Furthermore, no signs of pneumonia were detected in histopathological sections of the vaccinated and subsequent virus-challenged animals. In the absence of an effective antiviral drug against SARS-CoV-2, vaccines with good potency and safety will be needed to effectively establish immunity in the population. Based on the results presented here, a phase I clinical trial of CRCx is currently in progress.

Consent For Publication:

All authors consented for publication and approved the manuscript.

Conflict Interests:

The authors declare that they have no competing of interests.

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