Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Vaccine 39 (2021) 2280–2287

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Intranasal administration of a recombinant RBD vaccine induced protective immunity against SARS-CoV-2 in mouse

Yingying Du [a], Yuhua Xu [b], Jin Feng [b], Longbo Hu [c], Yanan Zhang [d], Bo Zhang [d], Weili Guo [b], Runming Mai [b], Liyun Chen [b], Jianmin Fang [c], Hui Zhang [a,*], Tao Peng [b,c,*]

[a] Institute of Human Virology, Zhongshan School of Medicine, Sun Yat-Sen University, Guangzhou, Guangdong, China
[b] Guangdong South China Vaccine, Guangzhou, China
[c] Sino-French Hoffmann Institute of Immunology, State Key Laboratory of Respiratory Disease, College of Basic Medical Science, Guangzhou Medical University, Guangzhou, China
[d] Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan, China

ARTICLE INFO

Article history:
Received 25 August 2020
Received in revised form 6 January 2021
Accepted 2 March 2021
Available online 5 March 2021

Keywords:
SARS-CoV-2
Intranasal administration
Mucosal immunity
Neutralizing antibody

ABSTRACT

The emergence of the global Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) pandemic underscores the importance of the rapid development of a non-invasive vaccine that can be easily administered. A vaccine administered by nasal delivery is endowed with such characteristics against respiratory viruses. In this study, we generated a recombinant SARS-CoV-2 receptor-binding domain (RBD)-based subunit vaccine. Mice were immunized via intranasal inoculation, microneedle-intradermal injection, or intramuscular injection, after which the RBD-specific immune responses were compared. Results showed that when administrated intranasally, the vaccine elicited a robust systemic humoral immunity with high titers of IgG antibodies and neutralizing antibodies as well as a significant mucosal immunity. Besides, antigen-specific T cell responses were also analyzed. These results indicated that the non-invasive intranasal administration should be explored for the future SARS-CoV-2 vaccine design.

1. Introduction

Owing to the rapid spread and high stability of the SARS-CoV-2, COVID-19 vaccines are in urgent need. The receptor-binding domain (RBD) of the SARS-CoV-2 spike (S) protein binds the human angiotensin-converting enzyme 2 (ACE2) receptor, which facilitates the cellular entry of the virus. Therefore, RBD is the primary target of neutralizing antibodies against the virus [1,2]. It has been demonstrated that, among a large number of SARS-CoV-1 candidate vaccines, the vaccines targeting RBD are more potent than those targeting the S-full-length protein [3–5] and can even avoid the risk of disease enhancement [6,7]. The antigen selection strategies used for SARS-CoV-1 are also applicable to the development of SARS-CoV-2 vaccine [8,9].

The success of a recently marketed intranasal-sprayed vaccine, FluMist™ [10], indicates that for vaccines against respiratory infectious diseases, vaccination through mucosal route may enhance the effect of the vaccine when compared to the traditional injected routes [11,12]. This is mainly because mucosal vaccines produce both systemic and local mucosal immune responses to scale up the war against the virus [13]. For example, intranasal administration induces large amounts of IgA antibodies at the site of inoculation, which neutralizes the viruses and excludes them from the primary infection [14–17]. Another advantage of intranasal vaccines that is critical for the COVID-19 pandemic is that the needle-free and noninvasive intranasal vaccine could be performed by simply trained personnel or even possibly performed via self-administration in a universal immunization campaign, thus requiring minimum medical resources. An adenovirus vectored vaccine expressing full-length SARS-CoV-2 protein administered via intranasal immunization was recently demonstrated to be protective in mice and rhesus macaques [18]. However, the immunogenicity of an intranasal immunized SARS-CoV-2 subunit-based vaccine remains largely unknown.

In the current study, mice were immunized with a recombinant SARS-CoV-2 RBD adjuvanted with Alhydrogel® (aluminium oxyhydroxide gel, alum) to investigate the immunogenicity of an intranasal delivered vaccine. The systemic immunity including the humoral, cellular response and the mucosal immunity induced via three different administration routes (intranasal inoculation, microneedle-intradermal injection, or intramuscular injection) were compared. Results showed that intranasal vaccination induced a better humoral response with a potent neutralizing

* Corresponding author.
E-mail addresses: zhangh92@mail.sysu.edu.cn (H. Zhang), peng_tao@gibh.ac.cn (T. Peng).

https://doi.org/10.1016/j.vaccine.2021.03.006
0264-410X/

activity, a balanced Th1/Th2 response, and a stronger mucosal immunity than the other two parenteral administrations.

2. Materials and methods

2.1. Cells, virus, plasmids

SF-α cells were supplied by Guangdong South China Vaccine Co., Ltd and maintained in the EXCELL-420 medium [19]. pFastBac™Dual and DH10Bac were from Invitrogen. MicronJet 600 Needle used as a device for intradermal administration was obtained from Nanopass Technologies Ltd. SARS-CoV-2 WIV04 (Genbank accession number: MN996528.1) was used in the live coronavirus neutralization assay. HIV-based SARS-CoV-2 pseudovirus allowing for single-cycle infection was constructed and used in the pseudo-coronavirus neutralization assay. hACE2, T7 RNA polymerase expression vectors (pRNP) co-transfected HEK293T cells and SARS-CoV-2 S protein, T7 promoter-luciferase expression vectors (pT7-LUC) co-transfected HEK293T cells were constructed in our laboratory. pRNP and pT7-LUC were obtained from Dr. Richard Longnecker of Northwestern University.

2.2. Reagents and antibodies

Alhydrogel® 2% was purchased from Brenntag Biosector, Denmark. Newborn Calf Serum (NBCS) were from Zhejiang Tianhang Biotechnology Co., Ltd. HRP-goat anti-mouse IgA alpha chain, HRP-goat anti-mouse IgG, HRP-goat anti-mouse IgG1, and HRP-goat anti-mouse IgG2a were purchased from Abcam. Mouse IFN-γ/IL-4 double-color ELISPOT kit was from Cellular Technology Limited.

2.3. Mouse immunization

Female BALB/c mice (aged 6 weeks; n = 6) were vaccinated three times at three-week intervals via the intranasal, intradermal, or intramuscular route, as shown in Fig. 1B. Ten μg of RBD was administered per mouse with or without 100 μg of alum via intranasal route under light isoflurane anesthesia, via microneedle-intradermal injection in the back after shaving, or through intramuscular injection in a right hindlimb. Each animal in the control groups (PBS groups and alum groups) received 100 μg of alum or the same volume of PBS. The volumes for injected (intradermal or intramuscular injection) and mucosal administration (intranasal inoculation) were adjusted to 50 μL and 25 μL per dose respectively. The study protocol was approved by the Institutional Animal Ethics Committee of Foshan Huamio Biotechnology Company Ltd.

2.4. Sample collection

Blood samples were collected before each immunization and 3 weeks after the final immunization by *retro*-orbital venous plexus puncture. The vaginal washing fluids were obtained by rinsing with 20 μL of PBS each time, three times a day on the 18th/19th/20th day after the final immunization. The nasal washes, bronchoalveolar lavage fluids and intestines were collected 3 weeks after the last immunization from 6 mice in each group. The nasal and lung lavage fluids were collected by 3 repeated washes with 200 μL of PBS, respectively. One hundred mg of feces-free intestine was cut into small pieces (1–2 mm) and placed in an EP tube containing 200 μL of PBS and rocked for 5 h. All the samples collected were centrifuged at 8000 × g for 10 min at 4 °C and the supernatants were stored at −80 °C till performing the ELISA assay.

2.5. Enzyme-linked immunosorbent assay

The IgG, IgG1, IgG2a, and IgA titers against the RBD or S protein of SARS-CoV-2 were determined using indirect ELISA. In brief, 200 ng/well of the RBD or S protein was added to coat 96-well microtiter plates and then incubated overnight at 4 °C. After blocking with 10% newborn calf serum (NBCS) for 1 h, serial two-fold dilutions of serum were added and incubated at 37 °C for 1 h. RBD-specific antibodies were detected using HRP-labeled goat anti-mouse IgG, IgG1, IgG2a, or IgA separately. Color was visualized by adding the substrate 3,3′,5,5′-tetramethylbenzidine (TMB), and the absorbance at 450 nm was read using an ELISA plate reader (iMARK, YQ-193). The endpoint titer was defined as the highest reciprocal dilution of serum, which was 2.1-fold of the background values. The titer of the sample was regarded as 0 when its OD value failed to reach 2.1 times the background OD value even if the titer may be less than the initial dilution.

2.6. Enzyme-linked immunospot assay

Next, IFN-γ- or IL-4-secreting cells were determined by using the ELISPOT mouse kit according to the manufacturer's instructions. Briefly, the IFN-γ/IL-4 antibodies were coated onto the plates at 4 °C overnight after pre-wetting the PVDF membrane with 70% ethanol. 4×10^5 spleen cells freshly isolated from mice were added in each well with RBD (50 μg/mL), without RBD or with ConA (50 μg/mL), and were incubated at 37 °C for 24 h. Wells containing no stimulus or containing ConA were considered as negative and positive controls, respectively. Plates were incubated with biotinylated IFN-γ or IL-4 at 37 °C for 2 h, followed by incubating with FITC-HRP and Streptavidin-AP substrate for 1 h. Finally, the substrate solutions were successively added to develop the IFN-γ or IL-4 spot. The numbers of spots representing the numbers of cytokines secreted were counted by CTL immunospot S6 Universal Analyzer (Cellular Technology Ltd., OH, USA).

2.7. Live coronavirus neutralization assay

The live SARS-CoV-2 neutralization assay of serum samples was performed in Animal Biosafety Level 3 Laboratory (ABSL-3) of Wuhan Institute of Virology, Chinese Academy of Sciences (CAS). After inactivation at 56 °C for 30 min, serum samples were serially diluted and co-cultured with the same volume of 50 PFU SARS-CoV-2 viruses (WIV04) at 37 °C for 1 h. The mixtures were subsequently transferred to the monolayers of Vero E6 cells in 24-well plates, then the plates were incubated at 37 °C for 1 h. The viral cytopathic effect (CPE) was monitored and the 50% neutralizing antibody titer (NT50) was presented as the highest dilution of the serum that reduced 50% of the CPE compared to the control well.

2.8. SARS-CoV-2 pseudovirus neutralization assay

For pseudovirus-based neutralization assay, hACE2-293T cells were seeded in 96 well-plates 18 h before infection. Serum samples were serially diluted and co-cultured with the same volume of pseudotype particles at 37 °C for 1 h. The mixtures were duplicated and added to the monolayers of hACE2 expressing cells in 96-well microtiter plates. Then, the plates were incubated at 37 °C in 5% CO_2. Forty-eight h later, the cells were lysed and measured using Promega Glomax (GloMax® Explorer). The results were presented as NT50.

2.9. Cell fusion assay

The cell–cell fusion model previously described [20] was adapted in this study. HEK293T cells co-transfected with hACE2 and pRNP were used as target cells. HEK293T cells co-transfected with SARS-CoV-2 S protein and pT7-LUC were used as effector cells. For SARS-CoV-2 S protein-mediated cell–cell fusion assay, effector cells, target cells, and the serum from immunized mice were co-cultured 72 h post-transfection. Luciferase activity was assessed after 24 h of incubation.

2.10. Statistical analysis

Data in this study were presented as the mean-value ± standard error of the mean (SEM). Comparisons between groups were performed by Student's T-test in GraphPad Prism 7.0 software. P values <0.05 were considered statistically significant.

3. Results

3.1. Intranasal administration of an RBD vaccine induced a rapid and significant RBD specific humoral response in mice

The recombinant RBD was expressed in insect cells infected with the recombinant baculoviruses carrying the RBD sequence. Following chromatography purification, the RBD was determined by SDS-PAGE and Western Blot, as shown in Fig. 1A. The RBD formulated with or without alum was immunized in mice 3 times at intervals of 3 weeks via the intranasal, intradermal, or intramuscular route (Fig. 1B). PBS and Alum were used as negative control vaccines, respectively. No abnormal or pathological changes were observed in mice during the vaccination process.

The mouse sera were collected at three time points, as shown in Fig. 1B, and SARS-CoV-2 RBD-specific IgG titers were determined. As shown in Fig. 2, after the first immunization, 2 out of 6 RBD + alum immunized mice became seropositive via intranasal administration. Following the second immunization, moderate levels of IgG antibodies were induced in all the mice that received RBD + alum intranasal or intradermal vaccination, whereas low levels of IgG titers were observed in only 3 out of 6 mice that experienced RBD + alum intramuscular vaccination. After the final immunization, higher antigen-specific lgG titers were elicited in RBD + alum intranasally immunized mice compared to that induced by the other two injected routes. In addition, only small amounts of RBD-specific IgG antibodies were detected in several mice after the final immunization of sole RBD via the two parenteral routes. The result of S-specific antibody responses after the last immunization is similar to that of RBD-specific antibody responses (Fig. S1). These results demonstrated that the rapid and significant IgG antibody response was elicited in intranasal immunized mice, whereas the gentle development of IgG antibody response was found in intramuscularly immunized mice before the

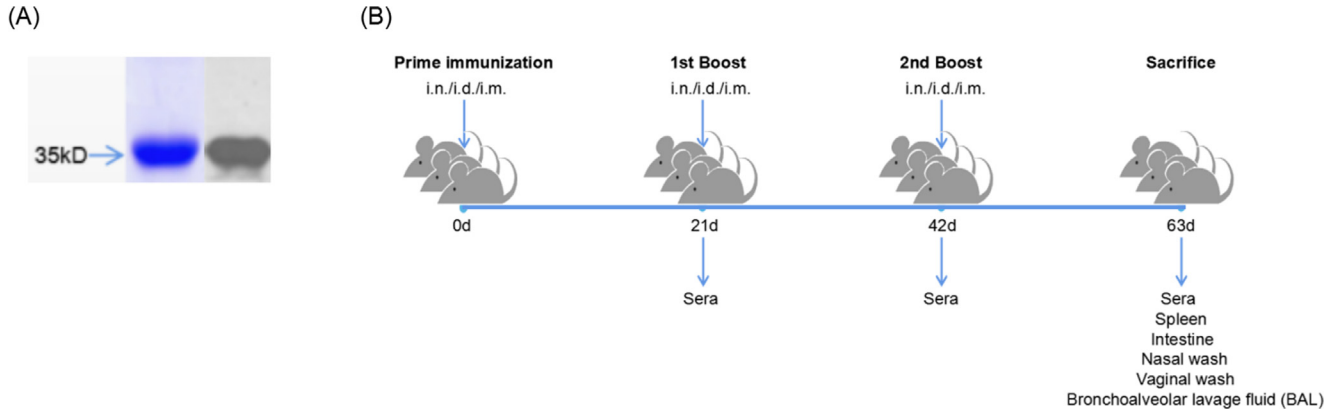

Fig. 1. Characterization of recombinant RBD and immunization schedule of mice. (A) Analysis of purified RBD. Rabbit anti-RBD polyclonal antibody was used for RBD detection. Left panel: SDS-PAGE of purified RBD. Right panel: Western blot of purified RBD. (B) Schematic of the vaccination schedule of mice. They were immunized three times at intervals of 3 weeks through intranasal immunization (i.n.), intradermal immunization (i.d.), or intramuscular immunization (i.m.). Blood samples were collected before each boost immunization and before sacrifice. Vaginal washes were collected for 3 consecutive days before sacrifice. Samples of spleens, nasal washes, bronchoalveolar lavage fluids, and intestines were collected at the time of sacrifice.

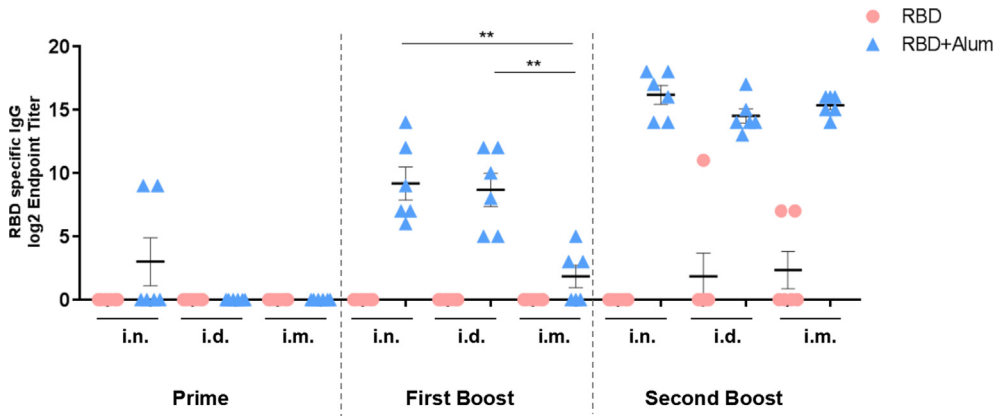

Fig. 2. SARS-CoV-2 RBD-specific IgG antibody response in mice after each immunization, as measured by ELISA. i.n.: intranasal immunization, i.d.: intradermal immunization, i.m.: intramuscular immunization. Results are expressed as the mean value ± standard error of the mean (SEM) of seven mice in each group. **0.001 < P < 0.01.

second boost. However, the antibody levels of the three groups became similar at the end of the study.

Cytokines secreted by Th1 cells mediate isotype switching to IgG2a, whereas cytokines secreted by Th2 cells mediate isotype switching to IgG1 [21]. The IgG1 and IgG2a ratio represents the balance between cellular immunity (Th1-skewed) and humoral immunity (Th2-skewed) [22]. Thus, the IgG subclasses (IgG1 and IgG2a) within the serum from immunized mice were further analyzed. After the last immunization, as expect, higher production of IgG1 than IgG2a indicating a Th2-skewed response, was seen in all groups co-administered with alum, consistent with previous studies [23,24]. However, it is worth noting that when vaccinated intranasally, alum was able to facilitate a mixed Th1/Th2 response compared to the other two parenteral routes (Fig. 3).

Overall, these results demonstrated that immunization with RBD adjuvanted with alum through intranasal route induced the fastest RBD-specific IgG antibody response. Furthermore, this route was able to elicit mixed Th1/Th2 response compared with the two other administration routes.

3.2. Intranasal administration of an RBD vaccine induced a prominent RBD specific mucosal response in immunized mice

To assess the production of RBD-specific sIgA at the mucosal site, nasal washes, bronchoalveolar lavage fluids (BAL), vaginal washes and intestines of the immunized mice were obtained after the final vaccination and tested by ELISA. Significant levels of IgA in nasal washes, BAL, and vaginal washes were observed in RBD + alum intranasally vaccinated mice but not in other mice (Fig. 4A, B, and C). The vaccination of RBD with or without alum in mice via any route elicited low levels of IgA antibodies in their intestines; at the same time, higher levels of IgA antibodies were induced through nasal administration (Fig. 4D). These results reflect the promising mucosal immunity induced by the nasal delivery of the RBD vaccine, which is consistent with previous studies [25,26].

3.3. IFN-γ and IL-4 producing T cells were elicited by intranasal immunization of an RBD vaccine in mice

After three rounds of immunization, lymphocytes isolated from spleens of the mice were stimulated with 50 μg/mL of the RBD. IFN-γ- and IL-4-secreting cells were determined by using the ELISPOT assay. Within the intranasal administration groups, limited IFN-γ and IL-4 secretions were detected in RBD + alum-adminis tered mice (Fig. 5A and B). The intradermal injection of RBD with alum displayed moderate production of IL-4 with limited secretion of IFN-γ (Fig. 5C and D). The IL-4 production induced in RBD + alum intramuscularly vaccinated mice was the highest among the three administration routes, although the IFN-γ production was still limited (Fig. 5E and F). Overall, these results demonstrated that the intranasal vaccination of the vaccine was capable of inducing T cell responses, but it was inferior to that induced by intramuscular administration. This is consistent with the findings of another study regarding adenovirus-based SARS-CoV-2 vaccine [18].

3.4. High levels of neutralizing antibodies were elicited by the intranasal immunization of an RBD vaccine in mice

Neutralizing antibody (Nab) titers against SARS-CoV-2 correlate well with protective efficacy to some extent [27]. To evaluate the protection provided by the vaccine, Nab titers of the serum collected after the second immunization were assessed using a SARS-CoV-2 virus (Strain: WIV04, GenBank Ref: MN996528.1) neutralization assay. As shown in Table 1, serum from the vaccine (RBD + Alum) intranasally immunized mice exhibited a neutralizing titer to 1:800 (NT50), which was twice as high as that induced in intradermally immunized mice. In contrast, limited neutralizing antibodies were detected in intramuscularly vaccinated mice. After the third immunization (2nd boost), the NT50 titers were assessed using the HIV-based SARS-CoV-2 pseudovirus neutralization assay due to limited resources of live virus. The results revealed that the vaccine administered by intranasal route induced the highest neutralizing antibody titers with the value (NT50) reaching over 1:3200. In comparison, the intradermal vaccination produced a weaker NT50 value, whereas intramuscular injection elicited an NT50 value inferior to that of intranasal vaccination but superior to that of intradermal vaccination. The NT50 value of serum from the convalescent donor cured from the infection was much lower than that of the serum from the RBD + Alum-immunized mice after the last immunization delivered via any administration route. These results indicated that the intranasal administration of the vaccine elicited a strong neutralizing activity against the virus.

3.5. Sera from intranasally immunized mice inhibited S protein-mediated membrane fusion

Fusion assay was used to determine the cell-fusion blocking activity of the sera samples. As shown in Fig. 6A, sera with high levels of RBD specific antibodies bind to the S protein, thus inhibiting the S protein-induced cell-fusion with reduced fluorescence. The results showed that 50-fold diluted serum from RBD + alum intranasally immunized mice exhibited a strong inhibition capacity in preventing about 40% of the cells from undergoing fusion,

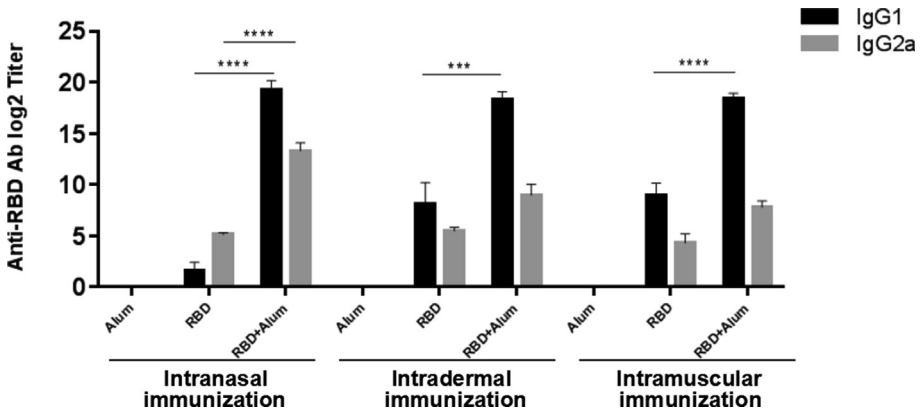

Fig. 3. SARS-CoV-2 RBD-specific IgG1 and IgG2a titers in mice 3 weeks after the last immunization. Results are expressed as the mean value ± standard error of the mean (SEM) of six mice in each group. ***0.0001 < P < 0.001; ****P < 0.0001.

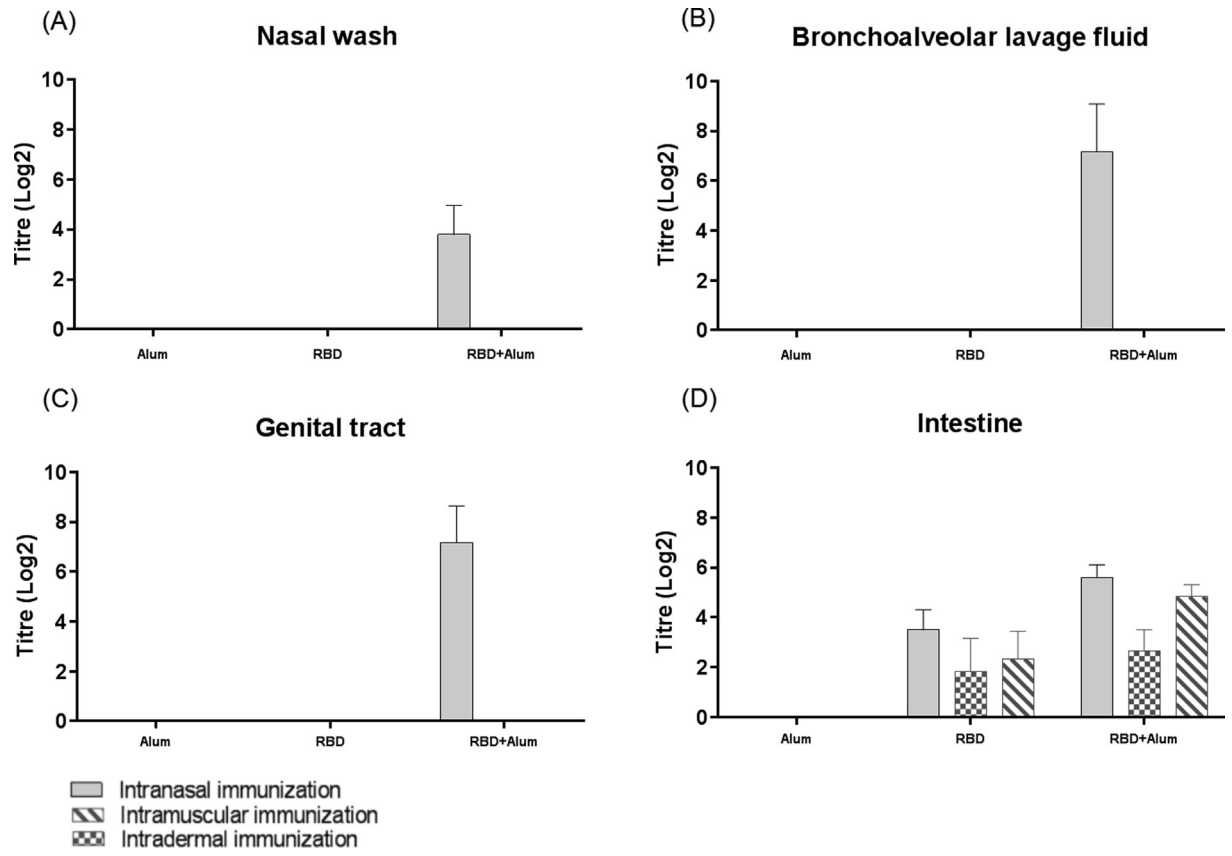

Fig. 4. SARS-CoV-2 RBD-specific secretory IgA titers in (A) nasal washes, (B) bronchoalveolar lavage fluids, (C) vaginal washes, and (D) intestines of mice 3 weeks after the last immunization. Results are expressed as the mean value ± standard error of the mean (SEM) of six mice in each group.

while the serum from intradermally vaccinated mice showed a weaker ability in limiting the cell–cell fusion. An extremely limited inhibition capacity was found through the intramuscular route (Fig. 6B). These results demonstrated that the intranasal administration of the RBD vaccine elicited a better cell-fusion blocking activity, which was in agreement with the results from the live virus and pseudovirus neutralization assays.

4. Discussion

In this study, we compared the immune responses induced by intranasal, intradermal or intramuscular immunization with a SARS-CoV-2 RBD subunit vaccine. The results indicated that the intranasal administration of the vaccine displayed an excellent profile in eliciting humoral and mucosal immunity. These findings suggest that an intranasal SARS-CoV-2 subunit vaccine warrants further development.

We were puzzled by the higher antigen-specific and neutralizing antibody responses after intranasal administration than after intramuscular administration during the first and second immunization. Whether this finding is due to the presence of a large number of antigen presenting cells (APCs) in nasopharyngeal-associated lymphoid tissue (NALT) induces different kinetics of antibody responses remains to be determined [28].

In addition to the potent humoral immunity, the intranasal RBD vaccine in our study also induced significant mucosal immunity on the mucosal surfaces of the nasal cavity, lung, genital tract, and intestine. The sIgA secreted by the mucosal B cells in the nasal cavity (upper respiratory tract) and lung (lower respiratory tract) might have formed the first-line defense against viruses that enter through the respiratory tract, thereby preventing them from invading the cells. These observations are consistent with the previous studies [25,26]. The mucosal B cells activated in the nasopharynx-associated lymphoid tissue (NALT) were then attracted by the epithelial CCL28/MEC and migrated to the distant effector sites, such as the intestine and the genital tract, where more IgA were produced [29–31]. According to some recent reports, SARS-CoV-2 infection may impair female reproductive function. The high level of IgA found in the vagina may be another potential advantage of the intranasal RBD vaccine [32,33].

An intranasal vaccine has many other unique advantages, including non-invasiveness and easy administration, which allow self-administration in non-clinical settings. These advantages are of importance for the COVID-19 pandemic, during which large crowds should be avoided and medical resources remain scarce. However, some limitations exist in the present intranasal vaccine forms. For instance, droplet vaccines require strict liquid packaging, while spray vaccines require the assistance of a special device. Dry powder is a recommended form, as it could be easily dispersed in water or saline and easily retain its viability and safety in room temperature, thereby avoiding the expensive cold-chain transportation [34].

Protein-based vaccines are known for their safety, but their immunogenicity is always compromised. Furthermore, adjuvants are needed to enhance their effectiveness. Aluminum salt adjuvants have been used clinically for nearly a century due to their effect on the enhancement of the immune response to antigen and their rare side effects; however, they are only limited to parenteral administration. Referring to intranasal use, the enhancement of the vaccine by alum was also evidenced by a few preclinical studies [35–37]. Therefore, in the current study, we

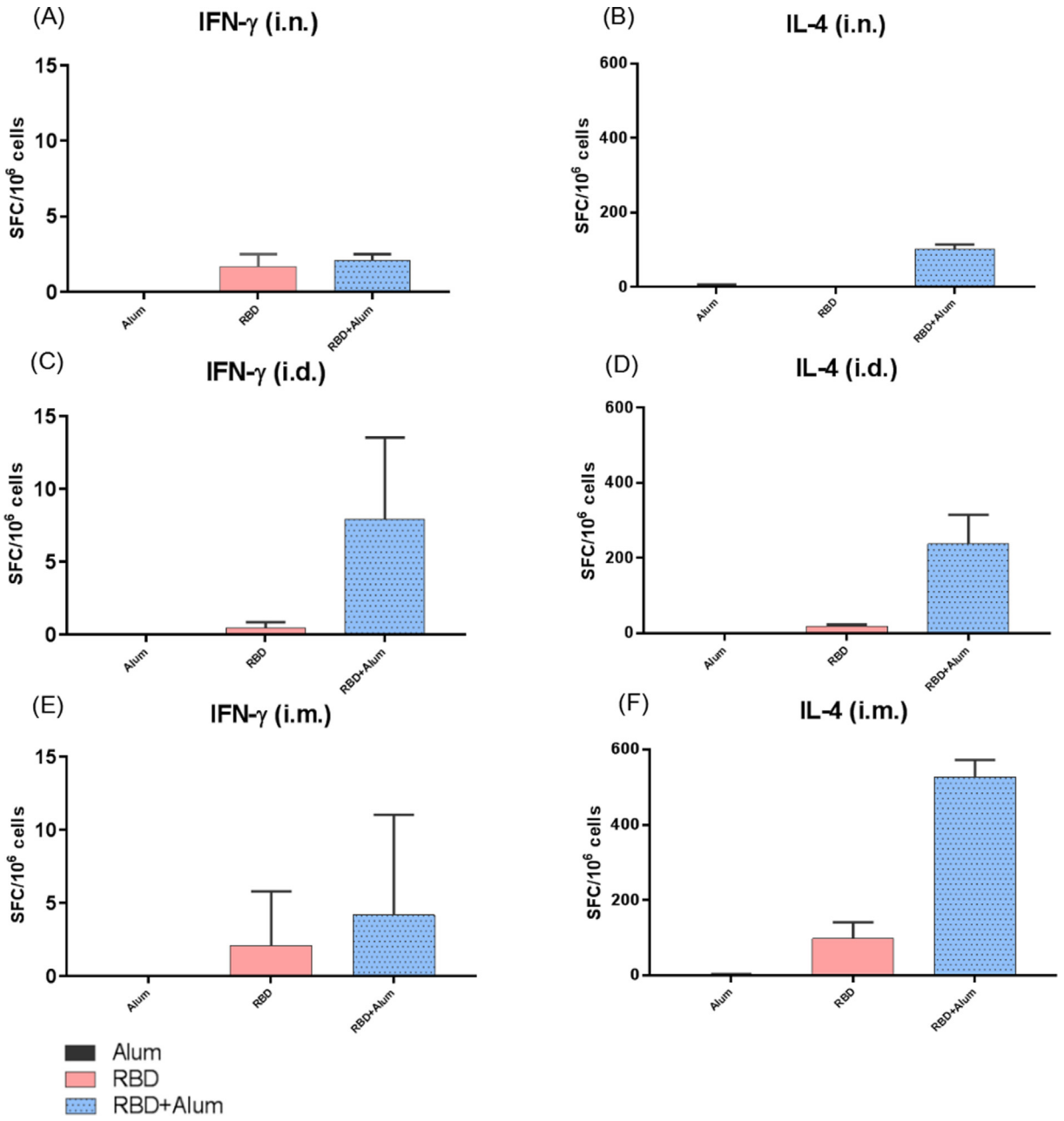

Fig. 5. ELISpot detection of IFN-γ producing cells (A, C, and E) and IL-4 producing cells (B, D, and F) in immunized mice. i.n.: intranasal immunization, i.d.: intradermal immunization, i.m.: intramuscular immunization. Results are expressed as spot-forming cells (SFCs) per 10^6 spleen cells.

Table 1
NT50 values of neutralizing antibodies against SARS-CoV-2 virus and SARS-CoV-2 pseudovirus in serum samples of mice after vaccination.

Administration route	Vaccine formulation	NT50 after 1st boost (SARS-CoV-2 neutralization assay)	NT50 after 2nd boost (SARS-CoV-2 pseudovirus neutralization assay)	NT50(SARS-CoV-2 pseudovirus neutralization assay)
Intranasal route	RBD + Alum	1: 800	1:3200–1:6400	
Intradermal route	RBD + Alum	1: 400	1:1600–1:3200	
Intramuscular route	RBD + Alum	<1: 10	1:3200	
Convalescent serum				1:400–1:800

chose alum as an adjuvant and found encouraging results. We also found that the use of alum during intranasal vaccination can better improve the effectiveness of the vaccine compared to other routes of administration. This may be due to the antigen adsorption properties of aluminum. In particular, the antigen was protected by the colloidal aluminum salt particles in the nasal cavity and then slowly released to be directly captured by the APC in NALT, thus extending the time and the scale of the immune response [25,37]. In addition, bacterial toxins and their derivatives, bacterial glycolipids, virosomes, plant-derived molecules, and cytokines seem to be the potential alternative adjuvants for intranasal protein-based vaccines [38].

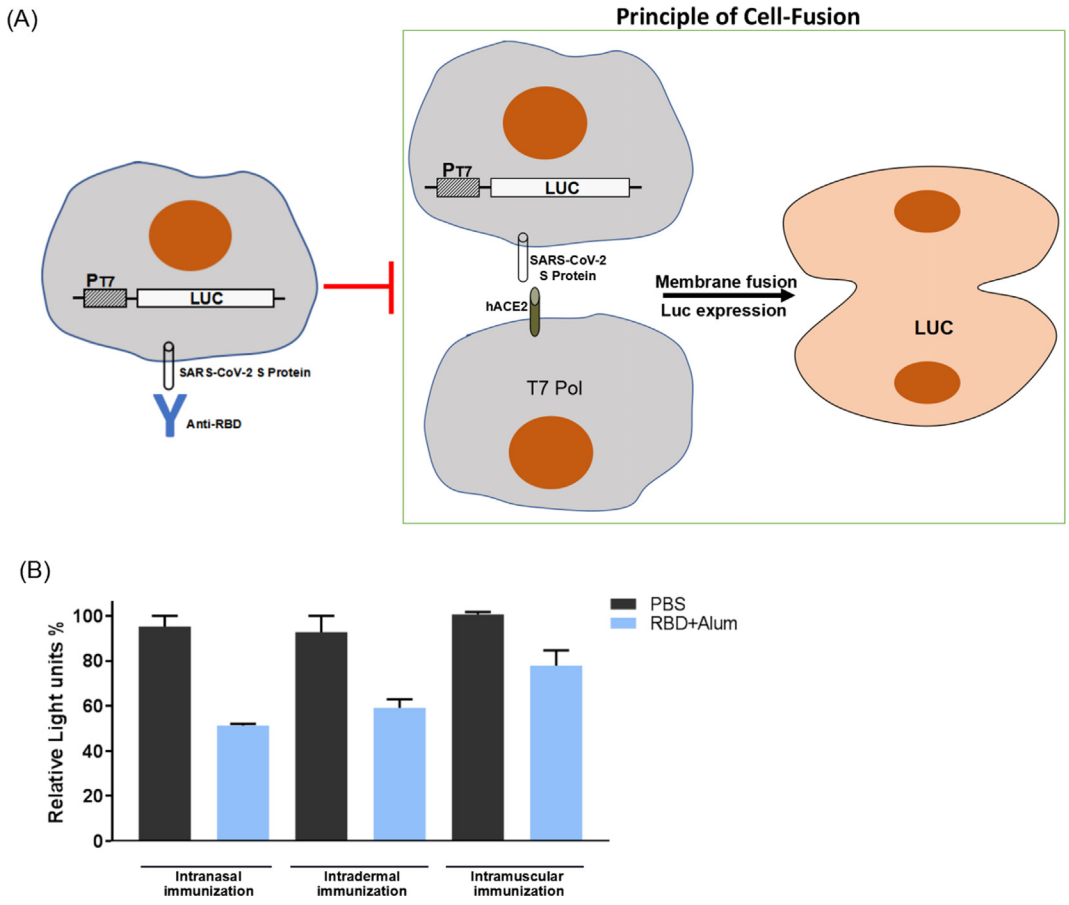

Fig. 6. Cell–cell fusion inhibition assay. (A) Principle of S protein-mediated cell–cell fusion assay. (B) Serum samples in each group were tested in duplicate. Data are presented as relative light units (RLUs) %.

In our study, the robust secretions of IL-4 and a Th2-bias response were observed following alum adjuvanted administration, which might raise concern about the immunopathology [39]. However, to date, no evidence for the disease enhancement of alum has been found in marketed vaccines or coronavirus vaccines in preclinical studies [6,40,41], including a recently reported SARS-CoV-2 vaccine [42]. Instead, alum could cause IL-4 secretion, which in turn, can lead to the priming and proliferation of B cells and the promotion of a potent antibody-mediated immunity [43–45].

Due to the limited resources of ABSL-3 animal laboratory, we have yet to perform the virus challenge experiments to show the protective efficacy in mice of our intranasal vaccine. Nevertheless, the nasal delivery of the recombinant RBD adjuvanted with alum represents a safe and promising strategy for SARS-CoV-2 vaccine development.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

We thank Dong Ding and Ziyao Luo for their excellent technical support. This study was supported by the COVID-19 prevention and control project of Guangzhou (Grant No. 202008040008), and the COVID-19 prophylaxis and treatment project of Guangdong Province (Grant No. 2020B111109003), the 111 Project (no. D18010), and Guangzhou Institute of Respiratory Health Open Project (Funds provided by China Evergrande Group, Project No. 2020GIRHHMS01).

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2021.03.006.

References

[1] Rogers TF, Zhao F, Huang D, Beutler N, Burns A, He WT, et al. Isolation of potent SARS-CoV-2 neutralizing antibodies and protection from disease in a small animal model. Science (New York, N.Y.); 2020; eabc7520. Advance online publication.
[2] Alturki SO, Alturki SO, Connors J, Cusimano G, Kutzler MA, Izmirly AM, et al. The 2020 pandemic: current SARS-CoV-2 vaccine development. Front Immunol 2020 Aug 19;11:1880. https://doi.org/10.3389/fimmu.2020.01880. PMID: 32973779; PMCID: PMC7466534.
[3] Du L, Zhao G, Li L, He Y, Zhou Y, Zheng BJ, Iang S. Antigenicity and immunogenicity of SARS-CoV S protein receptor-binding domain stably expressed in CHO cells. Biochem Biophys Res Commun 2009;384(4):486–90.
[4] Du L, Zhao G, Chan CC, Sun S, Chen M, Liu Z, et al. Recombinant receptor-binding domain of SARS-CoV spike protein expressed in mammalian, insect and E. coli cells elicits potent neutralizing antibody and protective immunity. Virology 2009;393(1):144–50.
[5] He Y, Zhou Y, Liu S, Kou Z, Li W, Farzan M, et al. Receptor-binding domain of SARS-CoV spike protein induces highly potent neutralizing antibodies: implication for developing subunit vaccine. Biochem Biophys Res Commun 2004;324(2):773–81.

[6] Tseng CT, Sbrana E, Iwata-Yoshikawa N, Newman PC, Garron T, Atmar RL, et al. Immunization with SARS coronavirus vaccines leads to pulmonary immunopathology on challenge with the SARS virus. PLoS ONE 2012;7(4):e35421.
[7] Jaume M, Yip MS, Kam YW, Cheung CY, Kien F, Roberts A, et al. SARS CoV subunit vaccine: antibody-mediated neutralisation and enhancement. Hong Kong Med J = Xianggang yi xue za zhi 2012;18(Suppl 2):31–6.
[8] Loes AN, Gentles LE, Greaney AJ, Crawford KHD, Bloom JD. Attenuated influenza virions expressing the SARS-CoV-2 receptor-binding domain induce neutralizing antibodies in mice. Viruses 2020;2020(10):3390.
[9] Liu Z, Xu W, Xia S, Gu C, Wang X, Wang Q, et al. RBD-Fc-based COVID-19 vaccine candidate induces highly potent SARS-CoV-2 neutralizing antibody response. Signal Transduct Target Ther 2020;2020(10):1038.
[10] Nils L. Recent progress in mucosal vaccine development: potential and limitations. Nat Rev Immunol 2012;12(8):592–605. https://doi.org/10.1038/nri3251.
[11] See RH, Zakhartchouk AN, Petric M, Lawrence DJ, Mok CP, Hogan RJ, et al. Comparative evaluation of two severe acute respiratory syndrome (SARS) vaccine candidates in mice challenged with SARS coronavirus. J Gen Virol 2006;87(Pt 3):641–50.
[12] See RH, Petric M, Lawrence DJ, Mok C, Rowe T, Zitzow LA, et al. Severe acute respiratory syndrome vaccine efficacy in ferrets: whole killed virus and adenovirus-vectored vaccines. J Gen Virol 2008;89(Pt 9):2136–46.
[13] Yusuf H, Kett V. Current prospects and future challenges for nasal vaccine delivery. Human Vac Immunotherapeutics 2017;13(1):34–45.
[14] Du L, Zhao G, Lin Y, Sui H, Chan C, Ma S. Intranasal vaccination of recombinant adeno-associated virus encoding receptor-binding domain of severe acute respiratory syndrome coronavirus (SARS-CoV) spike protein induces strong mucosal immune responses and provides long-term protection against SARS-CoV infection. J Immunol (Baltimore, Md. : 1950) 2008;180(2):948–56.
[15] Hu MC, Jones T, Kenney RT, Barnard DL, Burt DS, Lowell GH. Intranasal Protollin-formulated recombinant SARS S-protein elicits respiratory and serum neutralizing antibodies and protection in mice. Vaccine 2007;25(34):6334–40.
[16] Dhakal S, Renu S, Ghimire S, Shaan Lakshmanappa Y, Hogshead BT, Feliciano-Ruiz N, et al. Mucosal immunity and protective efficacy of intranasal inactivated influenza vaccine is improved by chitosan nanoparticle delivery in pigs. Front Immunol 2018;9:934.
[17] Kim S, Jang JE, Yu JR, Chang J. Single mucosal immunization of recombinant adenovirus-based vaccine expressing F1 protein fragment induces protective mucosal immunity against respiratory syncytial virus infection. Vaccine 2010;28(22):3801–8.
[18] Feng L, Wang Q, Shan C, Yang C, Feng Y, Wu J, et al. An adenovirus-vectored COVID-19 vaccine confers protecgtion from SARS-COV-2 challenge in rhesus macaques. Nat Commun 2020. https://doi.org/10.1038/s41467-020-18077-5.
[19] Yuhua Xu, Shuzhi Ma, Yangbo H, Fengying C, Liyun C, Dong D, et al. Virus-like particle vaccines for poliovirus types 1, 2, and 3 with enhanced thermostability expressed in insect cells. Vaccine 2019;37(17):2340–7. https://doi.org/10.1016/j.vaccine.2019.03.031.
[20] Zhu QC, Wang Y, Peng T. Herpes simplex virus (HSV) immediate-early (IE) promoter-directed reporter system for the screening of antiherpetics targeting the early stage of HSV infection. J Biomol Screen 2010;15(8):1016–20.
[21] Mosayeb R, Saeed S, Hanie K, Niknam HM. Lower levels of IgG1 in comparison with IgG2a are associated with protective immunity against Leishmania tropica infection in BALB/c mice. J Microbiol Immunol Infect 2017;50(2):160–6. https://doi.org/10.1016/j.jmii.2015.05.007.
[22] Zhao F, Qu J, Wang W, Li S, Xu S. The imbalance of Th1/Th2 triggers an inflammatory response in chicken spleens after ammonia exposure. Poult Sci 2020;99(8):3817–22. https://doi.org/10.1016/j.psj.2020.04.029.
[23] Reed SG, Bertholet S, Coler RN, Fried M. New horizons in adjuvants for vaccine development. Trends Immunol 2009;30(1):23–32.
[24] Baz M, Samant M, Zekki H, Tribout-Jover P, Plante M, Lanteigne AM, et al. Effects of different adjuvants in the context of intramuscular and intranasal routes on humoral and cellular immune responses induced by detergent-split A/H3N2 influenza vaccines in mice. Clin Vaccine Immunol: CVI 2012;19(2):209–18.
[25] Riese P, Sakthivel P, Trittel S, Guzmán CA. Intranasal formulations: promising strategy to deliver vaccines. Exp Opin Drug Deliv 2014;11(10):1619–34.
[26] Moreno-Fierros L, García-Silva I, Rosales-Mendoza S. Development of SARS-CoV-2 vaccines: should we focus on mucosal immunity?. Expert Opin Biol Ther 2020;20(8):831–6.
[27] Fan Wu, Aojie Wang, Mei Liu, Qimin Wang, Jun Chen, Shuai Xia et al. Neutralizing antibody responses to SARS-CoV-2 in a COVID-19 recovered patient cohort and their implications. Medrxiv; 2020.
[28] Pabst R. Mucosal vaccination by the intranasal route. Nose-associated lymphoid tissue (NALT)-structure, function and species differences. Vaccine 2015;33(36):4406–13. https://doi.org/10.1016/j.vaccine.2015.07.022. Epub 2015 Jul 18. PMID: 26196324.
[29] Brandtzaeg P. Function of mucosa-associated lymphoid tissue in antibody formation. Immunol Invest 2010;39(4–5):303–55.
[30] Wu HY, Russell MW. Nasal lymphoid tissue, intranasal immunization, and compartmentalization of the common mucosal immune system. Immunol Res 1997;16(2):187–201.
[31] Johansen FE, Baekkevold ES, Carlsen HS, Farstad IN, Soler D, Brandtzaeg P. Regional induction of adhesion molecules and chemokine receptors explains disparate homing of human B cells to systemic and mucosal effector sites: dispersion from tonsils. Blood 2005;106(2):593–600.
[32] Salamanna F, Maglio M, Landini MP, Fini M. Body localization of ACE-2: on the trail of the keyhole of SARS-CoV-2. Front Med (Lausanne) 2020;7:. https://doi.org/10.3389/fmed.2020.594495. PMID: 33344479; PMCID: PMC7744810594495.
[33] Yan J, Li R-Q, Wang H-R, et al. Potential influence of COVID-19/ACE2 on the female reproductive system. Mol Hum Reprod 2020;26:367–73.
[34] Marasini N, Kaminskas LM. Subunit-based mucosal vaccine delivery systems for pulmonary delivery – are they feasible?. Drug Dev Ind Pharm 2019;45(6):882–94.
[35] Isaka M, Yasuda Y, Kozuka S, Miura Y, Taniguchi T, Matano K, et al. Systemic and mucosal immune responses of mice to Aluminium-adsorbed or Aluminium-non-adsorbed tetanus toxoid administered intranasally with recombinant cholera toxin B subunit. Vaccine 1998;16:1620–6. PMID:9713937.
[36] Isaka M, Yasuda Y, Taniguchi T, Kozuka S, Matano K, Maeyama J, et al. Comparison of systemic and mucosal responses of mice to Aluminium-adsorbed diphtheria toxoid between intranasal administration and subcutaneous injection. Nagoya Med J 2001;45:5–15.
[37] Xu H, Ruwona TB, Thakkar SG, Chen Y, Zeng M, Cui Z. Nasal Aluminum (oxy) hydroxide enables adsorbed antigens to induce specific systemic and mucosal immune responses. Hum Vaccin Immunother 2017;13(11):2688–94.
[38] Wang S, Liu H, Zhang X, Qian F. Intranasal and oral vaccination with protein-based antigens: advantages, challenges and formulation strategies. Protn & Cell 2015;6(007):480–503.
[39] Graham BS. Rapid COVID-19 vaccine development. Science 2020;368(6494):945–6. https://doi.org/10.1126/science.abb8923.
[40] Zhou Z, Post P, Chubet R, Holtz K, McPherson C, Petric M, et al. A recombinant baculovirus-expressed S glycoprotein vaccine elicits high titers of SARS-associated coronavirus (SARS-CoV) neutralizing antibodies in mice. Vaccine 2006;24(17):3624–31.
[41] Roberts A, Lamirande EW, Vogel L, Baras B, Goossens G, Knott I, et al. Immunogenicity and protective efficacy in mice and hamsters of a β-propiolactone inactivated whole virus SARS-CoV vaccine. Viral Immunol 2010;23(5):509–19.
[42] Qiang Gao, Linlin Bao, Haiyan Mao, Wang Lin Xu, Kangwei Yang Minnan, et al. Development of an inactivated vaccine candidate for SARS-CoV-2. Science 2020;369(6499):77–81. https://doi.org/10.1126/science.abc1932.
[43] Jordan MB, Mills DM, Kappler J, Marrack P, Cambier JC. Promotion of B cell immune responses via an alum-induced myeloid cell population. Science (New York, NY) 2004;304(5678):1808–10.
[44] He P, Zou Y, Hu Z. Advances in aluminum hydroxide-based adjuvant research and its mechanism. Human Vac Immunotherapeutics 2015;11(2):477–88.
[45] Ghimire TR. The mechanisms of action of vaccines containing aluminum adjuvants: an in vitro vs in vivo paradigm. Springerplus. 2015;4:181. https://doi.org/10.1186/s40064-015-0972-0. PMID: 25932368; PMCID: PMC4406982.