ORIGINAL ARTICLE
Iran J Allergy Asthma Immunol
April 2022; 21(2):207-214.
Doi: 10.18502/ijaai.v21i2.9228

Modulation of Immune Responses against HA1 Influenza Vaccine Candidate by B-lymphocyte Stimulator Cytokine in Mice

Seyedeh Mahsa Bagheri Yazdi[1], Shahla Shahsavandi[1], Fatemeh Fotouhi[2], Majid Tebianian[1], and Mohammad Majid Ebrahimi [1]

[1] Razi Vaccine and Serum Research Institute, Agricultural Research Education and Extension Organization, Karaj, Iran
[2] Department of Influenza, Pasteur Institute of Iran, Tehran, Iran

Received: 28 June 2021; Received in revised form: 13 November 2021; Accepted: 5 December 2021

ABSTRACT

Utilizing subunit vaccines is one of the strategies to address influenza infection. Recent innovations have focused on high doses of vaccine antigens and immune enhancers or adjuvants to induce more robust and long-lasting immune responses. Here, an effect of the B cell-activating factor receptor (BAFF-R) to increase the magnitude and durability of immune responses of the recombinant HA1 (rHA1) protein against the H1N1 influenza virus was studied.

The HA1 protein and the effector domain of BAFF-R were expressed in the pET-28a (+) vector. Eight-week-old BALB/c mice were equally grouped into five groups (n=20). The 15 and 25 μg/μL of rHA1 were mixed with 2 μg/μL of rBAFF-R and injected three times for vaccinated groups. Three control groups received normal saline and two concentrations of rHA1. The ability of rBAFF-R in eliciting HA-specific antibody response and stimulating T lymphocyte proliferation to induce the cell-mediated immunity was assayed. Induction of protection was evaluated following the challenge with PR8 strain.

Analysis of immune responses showed that the co-administration of rBAFF-R with rHA1 boosted HI responses to the antigen in mice, whilst it was not able to promote the T cell proliferation responses against influenza. Compared to rHA1alone, the rBAFF-R/rHA1 generated efficient protection for the animals. There were no significant differences in eliciting the immune responses in mice immunized with the lower dose of rHA1 than that with the higher dose.

The data indicate the rBAFF-R can enhance the primary and memory immune responses to protect against influenza infection.

Keywords: B cell-activating factor receptor; Hemagglutinins; Immunity; Influenza A virus H1N1 subtype

INTRODUCTION

Since the marketing of the influenza vaccine in 1930, the disease is still a global health concern. Persons with pneumonia, myocardial infarction, diabetes, and immunocompromised individuals are

Corresponding Author: Shahla Shahsavandi, PhD;
Razi Vaccine and Serum Research Institute, Agricultural Research Education and Extension Organization, Karaj, Iran. Tel: (+98 26) 3457 0038, Fax: (+98 26) 3455 2194, E-mail: s.shahsavandi @rvsri.ac.ir

potentially at an increased risk of influenza-associated complications. Symptoms of the newly emergent SARS-CoV-2 are quite similar to influenza infection. Due to the unique feature of influenza viruses to increase Angiotensin-converting enzyme 2 (ACE2) expression and the uncertainty of the ending time of the SARS-CoV-2 pandemic, overlapping these respiratory infections poses international public health threat.[1,2] Therefore, controlling influenza infection during and after the current pandemic is of great importance.

Immunization with inactivated influenza vaccines leads to the stimulation of strain-specific antibody immunity and B cell memory. The protective responses to hemagglutinin (HA) glycoprotein that neutralize the virus are the best correlate for influenza immunization. Antibodies that bind to the head domain (HA1) have potent neutralizing activity, thus an HA1-based vaccine is capable of stimulating specific-reactive antibodies and inducing protective immune responses against influenza infection.[3-5] Drifts in the antigenic sites of HA affect the antibody response and lead to the emergence of immune-escape variants. The overall estimate of influenza vaccine effectiveness has varied between 10% and 60% over the last 15 years. Highly variable rates of vaccine efficacy are due to the match or mismatch between the vaccine strains and the circulating viruses and host immune responses.[6,7]

The lack of cross-reactivity against non-matching influenza strains may be resolved through the administration of a recombinant HA (rHA) subunit vaccine. Most of these antigens are poor immunogens.[3,8] The challenge is thus formulating the recombinant antigen with an adjuvant to reach adequate immunogenicity as well as to modulate immune cells for providing protection.[9] The licensed influenza adjuvants can potentially improve the quality of the B cell response but do not prime the cross-reactive T cell responses. The IL-1 family cytokines, Mx, HK-1, and type I interferon have been evaluated to generate memory B cell and T cell pools to improve the protective immunity of novel types of influenza vaccines. These components mainly orchestrate the adaptive immune responses by boosting the immunogenicity of an antigen, accelerating responses to a vaccine, dose sparing, and immunomodulation.[10-13] Differentiating the B cell subset into long-lived antibody-secreting plasma cells and memory cells expressing high-affinity antibodies is a critical process in the induction of adaptive immune responses. B-cell activating factor (BAFF), also known as B-lymphocyte stimulator cytokine, and its specific receptor (BAFF-R) are important regulators of the immune system and act as potent B-cell survival and co-stimulator of immunoglobulin production.[14,15] BAFF-R is encoded by the *TNFRSF13C* gene expressed mainly on B cells and a subset of T cells. In humans, BAFF-R deficiency is associated with very few circulating B cells, and very low IgM and IgG serum concentrations.[16] Structurally, BAFF-R is a single-pass type III transmembrane glycoprotein that contains a partial cysteine-rich domain in the ectodomain region.[17] The extracellular domain has some intrinsic capacity to activate canonical NF-κB and phosphoinositide-3-kinase-dependent signaling cascades, which are required for immature B cells to survive and maintain during the B cells' growth stages.[18-20] In the present study, we evaluated whether co-administration of BAFF-R and rHA1 may effective in improving immune responses against the H1N1 influenza virus in BALB/c mice.

MATERIALS AND METHODS

Expression of HA1 and BAFF-R

The study protocol was approved by the institutional ethical board (Ethics Board approval number: RVSRI.REC 98.022). Viral RNA from local H1N1 influenza (accession No: HQ419001) was extracted following biosafety protocols using the Ribospin™ (GeneAll; South Korea) kit. The coding sequence of HA1 corresponded to 980 bp was amplified with a primer set of F: 5′-ATGAAGGCAATACTAGTAGTTC-3′ and R: 5′-GTAAAAAGCACAAAATTGAG-3′ (Table 1) using reverse transcription-polymerase chain reaction (RT-PCR). The fragment was cloned into a pET-28a (+) vector (Novagen; Germany) between the *Bam*HI and *Hin*dIII restriction sites. Then the resulting plasmid was transformed into BL21 competent *E. coli* using the heat-shock method. The integrity of the extracted plasmid was confirmed by DNA sequencing. The rHA1 protein expression was induced by the addition of 0.4 mM isopropyl thiogalactoside (IPTG; Sigma-Aldrich) followed by incubation at 37 °C with shaking for 4 h. The harvested cells were sonicated for 5min (30 s sonication and 30s rest) and centrifuged. The protein was purified by using Protino Ni-TED (The Netherlands) and analyzed by 12% SDS-PAGE.

Datasets of BAFF-R peptide sequences were derived from the Uniprot (www.uniprot.org) and the domains were identified by the Simple Modular Architecture Research Tool (SMART). BAFF-R contains one extracellular cysteine-rich domain that starts at position 19 and ends at position 49, which serves for ligand binding as well as for self-assembly. Based on the data the primers F: 5′-CCAGTGCAATCAGACCGAG-3′ and R: 5′-CTCCAGGCTGCTTGTATGTC-3′ corresponded to 100 bp were designed so that the cDNA encoding the BAFF-R extracellular domain can be amplified. Total RNA was extracted from the blood sample of BALB/c mice; using TRIzol (Sigma-Aldrich) and recombinant BAFF-R (rBAFF-R) peptide was expressed in the same manner.

Table 1. One-step reverse transcription-polymerase chain reaction (RT-PCR) thermal cycling conditions for amplification of HA1 H1N1 influenza virus

RT-PCR cycle		Temperature	Time
1 cycle	Reverse transcription reaction	45°C	30 min
	Inactivation of RTase	94°C	5 min
30 cycles	Denaturation	94°C	20 sec
	Annealing	56°C	30 sec
	Extension	72°C	30 sec
	Final extension	72°C	5 min

Mice Immunization and Protection Trials

Before immunization, the concentration of rBAFF-R was adjusted to 2 μg/μL in PBS. The concentration of the HA1 purified protein was also adjusted to 15 and 25 μg/μL in PBS. The amounts of rHA1 vaccine were separately mixed with 2μg of rBAFF-R before injection. One hundred 8-week-old female BALB/c mice were randomly housed in five groups (n=20). Groups A and B were injected intramuscularly in the quadriceps muscle with the rHA1+rBAFF-R at 15 and 25 μg/μL, respectively. The controls include group C received normal saline, group D received rHA1 at 15 μg/μL, and group E received rHA1 at 25 μg/μL alone. Mice in each group were boosted two times at a regular interval of 14 days with the same vaccination regimen.

To determine the immune responses, blood samples were taken before vaccination and at a 2-weeks interval up to 91 days (end of the experiment). Hemagglutination inhibition (HI) assay was performed to determine HA-specific antibody levels. Briefly, sera were serially two-fold diluted in duplicate in a 96-well plate and incubated with 4 HA units of viral antigen for 30 min at room temperature. The mixture was incubated with 0.5% chicken red blood cell at room temperature for 45 min. Controls containing viruses or diluent alone were included on the plate. The inhibition of hemagglutination at the highest dilution of serum was considered the HI titer.[21] Samples were assigned as positive when their titer was ≥16.

The lymphocyte proliferation response to the PH-1 vaccine candidate was measured by using a 3-(4,5-dimethylthiazol-2-thiazolyl)-2,5-diphenyltetrazolium bromide, thiazolyl-blue (MTT) assay to evaluate the cellular immune responses.[11] Two weeks after the final immunization, the T-cell proliferation response to the rHA1+rBAFF-R was determined. The spleen cells obtained from the sacrificed mice were seeded in a 96-well plate at 2×10^5 cells/well in RPMI-1640 supplemented with 10% fetal bovine serum. The splenocytes were stimulated in the presence of rHA1 (1 μg/ml), phytohemagglutinin (PHA; 5 μg/mL), or RPMI-1640 culture media. Cells were for 72 h at 37°C and 5% CO_2 and then 10 μL of MTT (5 μg/mL final concentration) was added to each well. After a 4-h incubation, the absorbance was quantified by its measurement at OD_{540} and proliferative activity was expressed as a stimulation index (SI) calculated following formula:

SI=Mean OD of stimulated lymphocytes–Mean OD of blank/Mean OD of unstimulated lymphocytes

Induction of protection was assessed by determining the survival of mice following a challenge with the PR8 influenza virus. Four weeks after the final immunization, 10 mice per group were anesthetized and infected intranasal with 50 µL of 5 MID_{50} (mouse infectious doses of 50%) of PR8 strain. Mice were monitored daily for weight loss and survival for 14 days post-challenge. The immunization schedule is represented in Figure 1. All experimental animal procedures were carried out following the "Guidelines for the Care and Use of Laboratory Animals" approved by the Biomedical Research Ethics Committee.

Induction of protection was assessed by determining the survival of mice following the challenge with a mouse-adapted A/PR8/34 (H1N1) influenza virus. Tissue culture infectious dose 50% ($TCID_{50}$) titer of PR8 virus was determined by the inoculation of MDCK cell cultures. Groups of mice were anesthetized by intraperitoneal injection of ketamine-xylazine (ketamine 50 mg/kg body weight, xylazine 20 mg/kg body weight) and infected intranasally with 25 µL of infectious virus diluted in sterile PBS. The fifty-percent lethal dose (LD_{50}) was calculated according to the Spearman- Karber method. Four weeks after the final immunization, 10 mice per group were anesthetized and infected intranasally with 5 LD_{50} of the PR8 strain. Mice were monitored daily for weight loss and survival for 14 days post-challenge. The immunization schedule is represented in Figure 1.

Statistical Analysis

The data from immunized mice with those from control groups were analyzed by one-way ANOVA (SPSS ver. 21). Data are presented as means ± standard error (SD) of the mean as calculated from three independent experiments.

Significant differences were assigned when the *p-value* was <0.01.

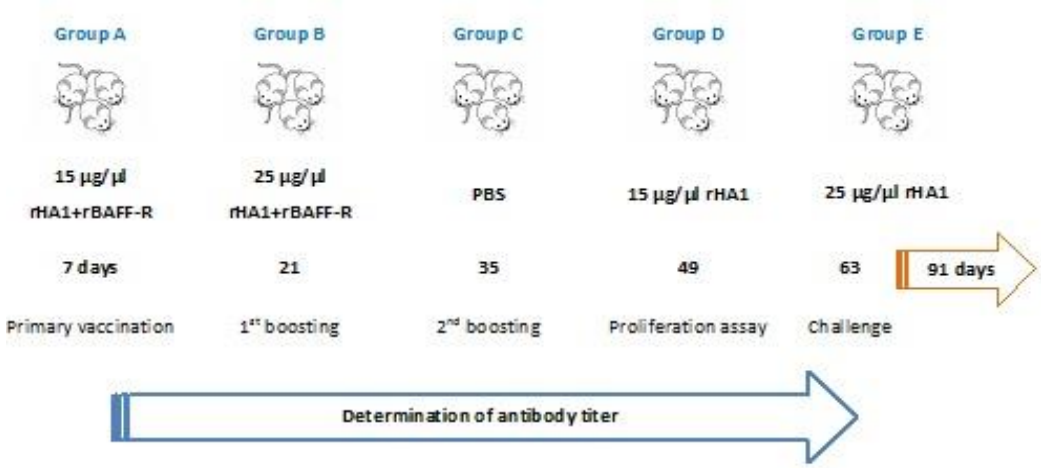

Figure 1. Representation of the immunization schedule of BALB/c mice. Groups A and B were primed with different formulations of recombinant HA1 (rHA1) +recombinant B cell-activating factor receptor (rBAFF-R) and then received two boosters. Other groups received the related samples. Two weeks after the second boosting, splenocytes obtained from three mice in each group were used in lymphocyte proliferation assay and two weeks later induction of protection against influenza was evaluated by a challenge. The hemagglutinin-specific antibody titers were determined by hemagglutination inhibition assay at two-week intervals up to the end of the experiment.

RESULTS

The genes encoding the HA1 and BAFF-R were amplified and cloned into the pET-28a (+) vector. The integrity of the recombinant vectors was confirmed by sequencing. The expression levels of the recombinant proteins were elevated by the addition of IPTG. The expression of HA1 protein was confirmed by SDS-PAGE and Western blotting at the expected size of 38 kDa. The yield was estimated at approximately 4.18

mg/mL for rHA1 and 4.34 mg/mL for rBAFF-R in the Bradford assay.

To evaluate the efficacy of rHA1+rBAFF-R in the induction of humoral immune responses in BALB/c mice, the collected sera were analyzed by HI assay (Figure 2). The results showed that the vaccinated mice in groups A and B developed statistically significant (p<0.01) higher antibody titers when compared with mice who received rHA1 alone. This indicates that rBAFF-R can stimulate measurable antibody responses against influenza. However, no significant differences were observed between the amounts of rHA1 in enhancing influenza virus-specific antibody responses. The post-booster antibody titers remained higher in the immunized mice, suggesting long-lasting antibodies were induced.

Two weeks after the last vaccination, the proliferation of lymphocytes stimulated either by HA1 or PHA was measured. The immunized mice with 15 µg/µL rHA1+rBAFF-R (3.72±0.23) and 25 µg/µL rHA1+rBAFF-R (3.78±0.21) showed better proliferation responses than rHA1 groups (Figure 3).

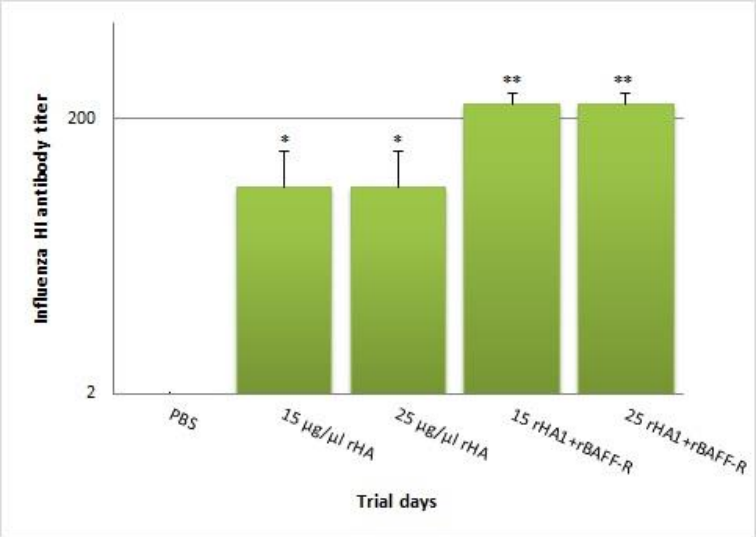

Figure 2. Influenza-specific antibody titers of mice groups immunized by recombinant HA1 (rHA1)+recombinant B cell-activating factor receptor (rBAFF-R) vaccine candidate measured by hemagglutination inhibition assay. Values are expressed as mean counts ± SE. *: significant difference (p<0.01) compared with PBS; **: compared with rHA1 alone. A titer >40 was considered positive.

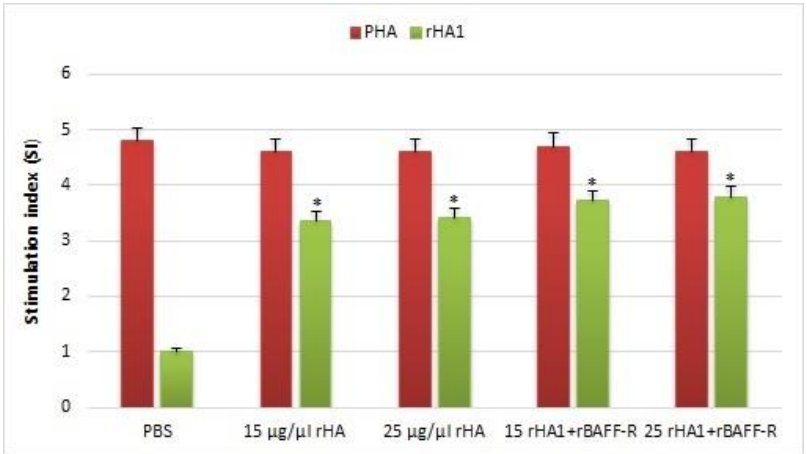

Figure 3. Assessment of splenocytes proliferation in response to recombinant HA1 (rHA1) in mice after the third immunization with rHA1+B cell-activating factor receptor (BAFF-R). *: statistically significant difference (p<0.01) between all immunized and control groups.

The results did not demonstrate a significant difference in SI in the adjuvanted rHA1 groups compared with the rHA1 group alone (p>0.01). The data revealed that BAFF-R could not improve the cellular proliferation level in immunization with the rHA1 antigen.

Four weeks after the final immunization, all groups of mice (n=10/group) were challenged intranasal with PR8 strain, and body weight and percent survival were recorded. The survival rate in both rHA1+rBAFF-R groups was 80% and the vaccinated mice have cleared the virus challenge infection efficiently. The differences between vaccinated and control groups were statistically significant (p<0.01). The progression of infection was indicated by a decrease in body weight (Figure 4). Mice in the rHA1+rBAFF-R groups experienced only a small and transient weight loss after the challenge. Mice immunized with rHA1 alone showed a 50% survival rate and a moderate weight loss with recovery to the initial level within 10 days. Infection of mice in the control group with PR8 strain was lethal, causing rapid and excessive weight loss (days 3-7) and 100% mortality within 7 days post-challenge.

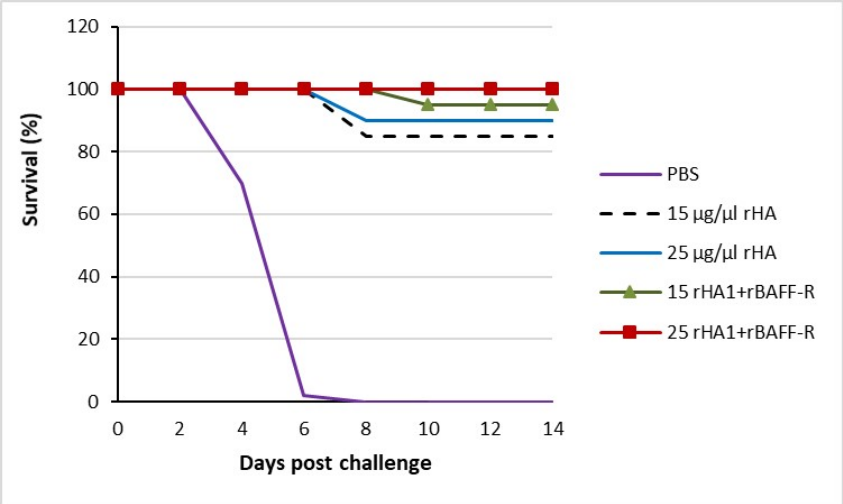

Figure 4. Comparison of survival percentage of BALB/c mice challenged with PR8 influenza virus. Since three mice out of each group were sacrificed for lymphocyte proliferation assay, this graph is based on the survival of the remaining mice after the challenge onwards.

DISCUSSION

The antigenic drift evidence of HA glycoprotein in the influenza virus genome and the emergence of immune escape variants have created a demand for new generations of vaccines. Although recombinant vaccines can induce immune responses against influenza infection, the scientific community is focused on new adjuvants to overcome the disadvantages of the vaccines. In the study, we evaluated exclusively the impact of BAFF-R on improving the immunogenicity of the rHA1 protein. Co-immunization of rBAFF-R with rHA1 resulted in enhanced higher levels of long-lasting HA-specific antibodies and protection against challenge infection in mice.

The HA protein represents an attractive target for vaccine development because of its important role in binding to sialic acid on target cells and virus entry. During these stages, HA especially at its globular head, which contains the majority of the immunodominant epitopes is accessible to B cell receptors and could potentially induce an antibody response.[6,22] The protective antibodies enhance by targeting the HA1 to antigen-presenting cells. With all the HA1 ability in the induction of neutralizing antibodies, the use of adjuvants is needed for increasing immunogenicity and stability of the antigen to improve protection against influenza. Over the last decade, studies have shown the ability of immunomodulators in increasing the protective potential of immunogens.[13,23] The molecules trigger an innate immune response with consequent

induction of cytokine production and dendritic cell (DC) activation. The activated T cells stimulate B cells to differentiate and produce antibodies eventually leading to the production of higher titers of antigen-specific antibodies. Recruitment of the DCs to the injection site, e.g., mice quadriceps muscles results in migration of the cells towards secondary lymph nodes, where the induction of T and B cell responses occurs. Mature DCs have a central role in the stimulation of immune responses by providing both antigen-specific and co-stimulation signals to T cells.[24] The cells also induce adequate antibody responses by delivering the viral antigen from the endosome to the cell cytosol for processing on antigen-presenting cells. The antigen presents on T helper cells and cytotoxic T lymphocyte (CTL) in association with major histocompatibility complex class I and class II molecules. This interaction is necessary for the development of both B cell and CTL memory, which is important in the induction of an adequate adaptive immune response.[25]

In association with migrating DCs, B cells are activated in the lymph nodes which act as major sites of B cell response induction following an influenza infection leading to higher titers of antigen-specific IgG. The same is targeted in vaccination against influenza, and stimulation and activation of B cells result in the production of circulating IgG antibodies that correlate with protection. The duration of antibody responses after utilizing inactivated influenza vaccine or rHA protein is a great challenge that may be resolved by the new vaccine adjuvants.[6,26] BAFF-R is responsible for the survival and promotion of B cells. During processing in circulating primary B cells, binding BAFF with the receptor activates several downstream pathways and induces a proteolytic cleavage that acts as a regulatory mechanism of BAFFR-dependent B cell survival and activation. The expression of BAFF-R is critical for enhancing immune responses and antiviral immunity through its responsibility for the survival and promotion of B cells.[15,16,19] Interactions between T cells, B cells, and DCs are necessary for the adequate and proper implementation of the adaptive immune response. BAFF-R promotes the interactions that lead to enhancement in antibody production. In our study, along with the increase in specific influenza antibody titers in mice that received rBAFF-R in combination with rHA1, a high level of the protective antibody was lasting 70 days after primary vaccination. It may follow the enhancing both effector and memory immune responses, which resulted in enhanced neutralizing antibody titers and improved levels of CTLs. Memory B cell responses are vital for protection against infections. The cells respond more rapidly than their naïve B cell precursors. Hence several strategies including boosting, increasing the concentration of antigen, and co-administration of adjuvants are still under evaluation to expand the IgG response. It has been found that the early antibody responses contribute to the optimal formation of B cell memory through IgG and BAFF. The BAFF-induced IgG secretion is mediated by inducing the expression of CD21 and CD23 on the B cell surface. Signals through this regulator molecule promote B-cell maturation and survival and also induce PI3K/Akt/mTOR pathway that involves in antibody production.[18-20]

Our study highlights the adjuvant effect of BAFF-R for immunization with rHA1 in the antibody response. Overall, the increased immunogenicity of the adjuvanted recombinant protein was because of the presence of BAFF-R which modulates antibody responses following regulation of B cell response. The rHA1 alone could stimulate the T lymphocyte proliferation responses but its formulation with BAFF-R was not efficacious in improving the cell-mediated immunity response evaluated; using lymphocyte proliferation assay. In licensed influenza vaccines, the only neutralizing antibodies can ascertain correlates of protection, which are not usable for cell-mediated immunity. Hence, efforts should be made to identify the correlation between the immune responses, neutralizing activity, and protection, particularly for next-generation influenza vaccines.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ACKNOWLEDGEMENTS

This study is the result of the MS thesis in medical virology supported by the Razi Vaccine and Serum Research Institute.

REFERENCES

1. Bai L, Zhao Y, Dong J, Liang S, Guo M, LiuX, et al. Coinfection with influenza A virus enhances SARS-

CoV-2 infectivity. Cell Res. 2021;31(3):395–403.
2. Cuadrado-Payán E, Montagud-Marrah M, Torres-Elorza M, Bodro M, Blasco M, et al. SARS-CoV-2 and influenza virus co-infection. Lancet. 2020;6736(20):31052-7.
3. Padilla-QuirarteHO, Lopez-Guerrero DV, Gutierrez-Xicotencat L, Esquivel-Guadarrama F. Protective antibodies against influenza proteins. Front Immunol. 2019;10:1677.
4. Watanabe A, McCarthy KR, Kuraoka M, Schmidt AG, Adachi Y, Onodera T, et al. Antibodies to a conserved influenza head interface epitope protect by an IgG subtype-dependent mechanism. Cell. 2019;177(5):1124-35.
5. Mallajosyulaa VVA, Citronb M, Ferrarac F, Lub X, Callahanb C, et al. Influenza hemagglutinin stem-fragment immunogen elicits broadly neutralizing antibodies and confers heterologous protection. PNAS. 2014; 111(21):13133-8.
6. Krammer F. The human antibody response to influenza A virus infection and vaccination. Nat Rev Immunol. 2019;19(3):383–97.
7. Garten RJ, Davis CT, Russell CA, Shu B, Lindstrom S, et al. Antigenic and genetic characteristics of swine-origin 2009 A (H1N1) influenza viruses circulating in humans. Science. 2009;325(5937):197-201.
8. Soema PC, Kompier R, Amorij JP, Kersten GF. Current and next generation influenza vaccines: Formulation and production strategies. Eur J Pharm Biopharm. 2015;94(9):251-63.
9. Fox, CB, Kramer, RM, Barnes, L, Dowling, QM, Vedvick, TS. Working together: interactions between vaccine antigens and adjutants. Ther Adv Vaccines. 2013;1(1):7-20.
10. Kayamuro H, Yoshioka Y, Abe Y, Arita S, Katayama K, Nomura T, et al. Interleukin-1 family cytokines as mucosal vaccine adjuvants for induction of protective immunity against influenza virus. J Virol. 2010;84(24):12703-12.
11. Soleimani S, Shahsavandi S, Madadgar O. Improvement influenza HA2 DNA vaccine cellular and humoral immune responses with Mx bio adjuvant. Biologicals. 2017;46(3):6-10.
12. Dehghan A, Shahsavandi S, Jabalameli L. Improvement efficacy of influenza nanovaccine in combination with hemokinin-1 molecular adjuvant. Avicenna J Med Biotechnol. 2018;10(4):208-13.
13. Thompson AL, Staats HF. Cytokines: The future of intranasal vaccine adjuvants. Clin Dev Immunol. 2011;2011:289597.
14. Nga, LG, Macka, CR, Mackay, F. The BAFF/APRIL system: life beyond B lymphocytes. Mol. Immunol. 2015;42(7):763-72.
15. Rodig, SJ, Shahsafaei, A, Li, B, Dorfmana DM. BAFF-R, the major B cell–activating factor receptor, is expressed on most mature B cells and B-cell lympho proliferative disorders. Human Pathol. 2005;36(10):1113-9.
16. Smulski CR, Eibel H. BAFF and BAFF-Receptor in B cell selection and survival. Front Immunol. 2018;9:2285.
17. Karpusas, M, Cachero, TG, Qian, F, Boriack-Sjodin, A, Mullen, C, et al. Crystal structure of extracellular human BAFF, a TNF family member that stimulates B lymphocytes. J Mol Biol. 2002;315(5):1145-54.
18. Zhang, F, Song, SS, Shu, JL, Li, Y, Wu, YJ, et al. BAFF upregulates CD28/B7 and CD40/CD154 expression and promotes mouse T and B cell interaction in vitro via BAFF receptor. Acta Pharmacol Sin. 2016;37(8):1101-9.
19. Rolink, AG, Tschopp, J, Schneider, P, Melchers, F. BAFF is a survival and maturation factor for mouse B cells. Eur J Immunol. 2002;32(7):2004-10.
20. Guan, NGL, Sutherland, APR, Newton, R, Qian, F, Cachero, TG, et al. B Cell-activating factor belonging to the TNF family (BAFF)-R is the principal BAFF receptor facilitating BAFF costimulation of circulating T and B cells. J Immunol. 2004;173(2):807-17.
21. Manual for the laboratory diagnosis and virological surveillance of influenza. WHO Global Influenza Surveillance Network. World Health Organization.2011. https://apps.who.int/iris/handle/10665/44518.
22. Angeletti D, Kosik I, Santos JJS, Yewdell WT, Boudreau CM, Mallajosyula VVA, et al. Outflanking immunodominance to target subdominant broadly neutralizing epitopes. PNAS. 2019;116(27):13474-9.
23. Nicholls EF, Madera L, Hancock RE. Immunomodulators as adjuvants for vaccines and antimicrobial therapy. Ann N Y Acad Sci. 2010;1213(23):46-61.
24. Waithman, J, Mintern, JD. Dendritic cells and influenza A virus infection. Virulence. 2012;3(1):603-8.
25. Allenspach, EJ, Lemos, MP, Porrett, PM, Turka, LA, Laufer, TM. Migratory and lymphoid-resident dendritic cells cooperate to efficiently prime naive CD4 T cells. Immunity. 2008;29(5):795-806.
26. Steel, J, Lowen, A, Wang, TT, Yondola, M, Gao, Q, et al. Influenza virus vaccine based on the conserved hemagglutinin stalk domain. MBio. 2010;1:e00018-10.