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Research Article

Advances and Prospects in MRNA Vaccines: Modern Horizons Versus New Emerging COVID-19, (SARS-CoV-2)

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ABSTRACT

Over the past two decades, plenty of measures have been done in mRNA based technologies for candidates for vaccines. According to pre-clinical and clinical trials, mRNA vaccines not only are able to make long- term immune responses in both humans and animal models, but they are safe. Moreover, they have high potency for rapid improvement and the process of their manufacture is cost-effective; nevertheless, delivering of mRNA vaccines had faced some difficulties due to their instability and inefficient in vivo. During the write of this article, clinical trials are currently administrated by monster corporations vaccine producing around the globe, yet no remedy approach has been proved for new emerging SARS-CoV-2. Vaccine is taken into account as a major section in prevention of emerging viral diseases. In summary, this review discusses the two main classes of mRNA vaccines: conventional non-amplifying and self-amplifying mRNA. It summarizes the initial clinical studies and outlines the delivery system for mRNA vaccine based on technology. Finally, this review highlights a new perspective of mRNA vaccine based on technology against SARS-CoV-2 (COVID-19).

KEYWORDS

SARS-CoV-2, mRNA, vaccines, delivery system, COVID-19.

INTRODUCTION

Coronaviruses (CoVs) cause widespread respiratory, gastrointestinal, and central nervous system diseases in humans and other animals, and threatens human and animals health. Coronaviruses are able to adapt to new environments through mutations and therefore programmed to be more efficient in terms of host domain and tissue tropism. Therefore, human and animal health threats due to coronaviruses will be constant and long-term, so by studying and researching coronaviruses and controlling their prevalence, important and beneficial consequences for global health and economic stability can be achieved. The RNA group of viruses is classified into three orders that include the order Nidovirales, which is further classified into four families: the Coronaviridae, Arteriviridae, Mesoniviridae, and Roniviridae. The family Coronaviridae is further divided into two subfamilies: Coronavirinae and Torovirinae. The Coronavirinae subfamily includes four genera of viruses (Alphacoronaviruses, Betacoronaviruses, Gammacoronaviruses, and Deltacoronaviruses), which have been grouped primarily based on serology and phylogenetic clustering (divisions based on the habitat/genetic relatedness) [5]. The detailed classification along with the origin of severe acute respiratory syndrome associated coronavirus 2 (SARS-

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CoV-2) is depicted in Figure 1. Among genera, alpha and beta-coronaviruses, mammals, gamma-coronaviruses, avian and Deltacronaviruses infect both mammals and avian.

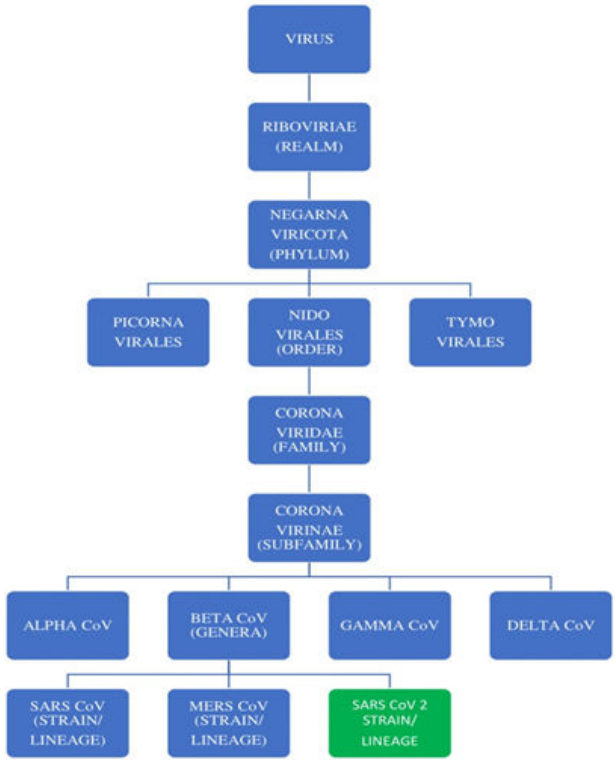

Figure 1

The classification of the RNA group of viruses and the origin of SARS CoV-2

From 2002 to 2003, coronavirus (acute respiratory syndrome (SARS-CoV)) infected 8,000 people, with a mortality rate of about 10%. Also, since 2012, the Middle East Respiratory Syndrome (MERS-CoV) has infected more than 1,700 people with a mortality rate of 36%. Recently, with the advent of a new Chinese epidemic in late 2019, history has repeated . New coronavirus called (SARS-CoV-2) that causes acute respiratory disease. This virus is enveloped and has a non-segmented, single-stranded, positive-sense ribonucleic acid (ssRNA+) as their nuclear material. Their genome is packgeded with nucleocapsid proteins (N-Proteins) inside a helical capsid. At the surface of the viral coat, there are structural proteins, including Membrane proteins (M) and Envelope protein (E), which play a role in assembling the virus in the host cell, as well as spike proteins(S), which act as ligands for Binding to the cell receptor (the angiotensin converting enzyme (ACE2)) for entering the host cell. Protein S In addition to its role in binding and entering the host cell, it can be a determinant of tissue tropism, and a major inducer of stimulation of the humoral and cellular immune response (TCD4 + and TCD8 +), so vaccine design on this protein is very important. These viruses are important pathogens in humans and other vertebrates after the outbreak of SARS (SARS-CoV) in 2002 in China. These viruses can infect the respiratory tract of humans and other animals. The prevalence of SARS and MERS, both of which appear to have originated in bats, suggests the possibility of transmitting these viruses from animal to human and from human to human. The rapid and widespread outbreak of SARS-CoV-2 and also 2 to 3% mortality in the world, indicates a serious threat for global health. The main way of spreading this disease is through respiratory droplets, which are spread in the environment with the cough of the infected person. Symptoms of SARS-CoV-2 include fever, fatigue, and dry cough, which are the most common symptoms of Covid-19. These symptoms are usually mild. Some infected persons may not experience any of the symptoms of the disease. Most patients (about 80%) recover without the need for special treatment. Approximately one in six people with Covid-19 becomes severely ill and suffers from shortness of breath. The elderly and people with underlying medical conditions such as high blood pressure, heart disease or diabetes are more likely to have the disease worse. In addition to these symptoms, some patients may have other symptoms such as pain and bruising, nasal congestion and even numbness and runny nose, sore throat, diarrhea and red eyes. As regards, there is currently no definitive cure for COVID-19, it is necessary to develop treatment and prevention methods and to make an effective vaccine.

mRNA-based vaccine technologies have made a new era in vaccination. Although the formulation, stability, and delivery of DNA vaccines are better than those of mRNA vaccines. The mRNA vaccines are a promising approach to against infectious diseases. The mRNA vaccines have significant advantages including high potential, economical production cycles, and they are safe without any noticeable toxicities. In other words use of mRNA has several useful features compared to other vaccines such as DNA vaccines or conventional vaccines: 1) Safety, there is considered a non-integrating and non-infectious platform and there are not any potential risks for mutagenesis as well as infection, 2) mRNA is a biodegradable molecule, 3) The mRNA modifications lead to increasing the stability and translation of mRNA, and 4) They are the smallest genetic vectors that can be used repeatedly without inducing immunity as seen in viral vectors and the rapid immune responses are directed toward only the selected antigens of interest from the pathogen. Woff et al. (1) was the first to report that mRNA were effective in transmitting genes directly into the skeletal muscle of a mouse resulted in expression of the encoded protein.

Recently, two types of mRNA vaccines have been developed (Fig. 1.): conventional mRNA vaccines and self-replicating mRNA vaccines derived from positive-sense RNA viruses. Conventional mRNA vaccines, in the simplest form, include ORF for the target antigen, UTR regions at both ends, and a poly A tail and express the target antigen, transiently. The self-amplifying mRNA derived from positive-sense ssRNA viruses, especially in alphaviruses (2). There are several steps in producing mRNA vaccines as follows. The in vitro transcription involves; 1) linear expression cassettes encoding mRNA as a vaccine template, 2) an RNA polymerase to generate mRNA from the template, and 3) triphosphate nucleotide as a backbone of mRNA synthesis. The cap structure associates with enzymatic function at the end

of the reaction, or as a synthetics analog that the cap is added to it. Finally, through adding a poly A tail, a mature mRNA sequence is created. After transfection, the mRNA vaccines also direct RNA synthesis, depending on the RNA polymerase complex, produces several copies of the antigen-encoding mRNA, and the exogenous gene is overexpressed when they enter the host cytoplasm. These vaccines, which have the ability to self-replication according to the mechanism mentioned above, are called self- replicating mRNA vaccines. The expression period in self-amplifying RNA molecules can extend to almost three months in vivo (3). One of the advantages of self-replicating RNA vaccines is that they produce their adjuvants in dsRNA form. They are about 9 to 11 kilobase length and are produced from a DNA template via similar methods to normal mRNA. Whereas the DNA vaccines must pass through the cytoplasmic and nuclear membranes. Then, it has to be transcribed to mRNA, then return from the nucleus to the cytoplasm to begin translation. Due to significant advances in RNA-based vaccines and their numerous applications, a large number of mRNA vaccines have entered to clinical phase studies. Over the past two decades, mRNA vaccines have been developed to prevent infectious diseases. There is a need for this vaccine in the good manufacturing practices (GMP) field although its clinical trials face limitations. Several RNA-based vaccines have been used to develop influenza vaccines that not only effect on homologous genes but also effect on heterologous genes (4). For example, its effective results on embryonic eggs have led to the production of long- persistence antigens in mammalian cells. There are some examples of mRNA vaccines against infectious diseases in clinical trial phases: the first one is the influenza virus with a route of nucleoside-modified viral antigen mRNA intramuscular injection (i.m.) which is ongoing in-phase (I) that Moderna Therapeutics is its sponsor. The second is HIV-1 with the type of electroporated dendritic cells (DC EP) with autologous viral antigen and CD40L mRNAs subepidermal injection (i.d.) completed in both (I/II) clinical phases with sponsoring of McGill University Health Centre. The third example is the rabies virus with a route of RNA active rabies virus glycoprotein (RABV-G) antigen mRNA (i.m., i.d.) which is now active. The fourth one is the zika virus with a route of nucleoside-modified viral antigen mRNA (i.m.) in both (I/II) clinical phases and now it is recruited (5). The last one is Metastatic Melanoma with the rout of (i.d.), and active Melan-A, tyrosinase, gp100, MAGE-A1, MAGE-A3, surviving and there is a rise in tumor specific T-cell responces in some patients regarding to phase of I and II (4). No mRNA vaccine has yet offered in the global markets so far, thus it probably needs more time to gain quality standards as well as safety evaluation. The Moderna corporation has been experienced a SARS-CoV-2 mRNA vaccine with characteristic of mRNA-1273, encoding S protein, both on animals and clinical trials batch. Many scientists and researchers, including Corbett et al., (2020); Jackson et al., (2020); Mulligan et al., (2020); Lackzko et al, (2020); McKay et al., (2020); Erasmus et al., (2020) etc.) have worked and published on mRNA vaccines based on COVID-19. In this present study, we will discuss several strategies for developing mRNA vaccines based on current understandings of various coronaviruses, particularly SARS-CoV-2.

Delivery and formulation

Delivery routes have an important role in mRNA vaccines to reach their full potential since naked RNA is very large and it has the potential to be degraded by nucleases. According to reports, less than 1 in 10,000 molecules of naked mRNA can be up taken by cells, meaning that this system concentrates on improvement, and identification of RNA transport to cells and mRNA delivery methods, depending on the non-viral delivery of small interfering RNA (siRNA). There are diverse important delivery routes such as encapsulating mRNA molecules into particles and others employing positively charged polymers for binding to RNA through charge interactions (2). Carriers usually have to pass through the target cell membrane by endocytosis. While it depletes its mRNA cargo into the place of translation (cytosol) it has to escape the endosome where pH is approximately 5-6. It is delivery routes that can not only stimulate the innate immune system and make a synergistic adjuvant effect, but it can impact on quality and quantity of local gene expression templates (6). It seems that this delivery route may be also used for SARS-CoV-2 based on mRNA vaccines.

Naked mRNA has been done successfully in vitro for immunization, especially for targeting some antigens in cells. The simplest strategy for prescribing intramuscular injection (i.m.) is naked mRNA which was initially shown to express the reporter gene in mice due to the impact of naked mRNA on i.m. confirmed. Also, naked mRNA injection is given subcutaneously (s.c.) and subepidermal (i.d.) in addition to i.m., it was confirmed and was effective in wound healing and curing of various skin diseases by expressing different proteins in the skin (7). According to the influenza test, delivery of naked mRNA elicited potent CD4+ and CD8 +T cell responses in mice and repeated intramuscular injection (i.n.) injection with modified mRNA cause priming antigen-specific CD4+ and CD8+ T cells (4, 8). Diethylaminoethyl (DEAE) Dextran was the first polymer testing as a delivery system for IVT mRNA.

According to the recent investigation, transcription from mRNA in vitro is one of the attractive purposes in drug and vaccine development. There are two different therapeutic approaches for mRNA vaccines: in vitro and ex vivo: (1) injecting direct mRNA and, (2) transfection or cellular mRNA delivery from patients. An attractive purpose of using self-replicating RNA viruses is that it has raised therapeutic efficiency in gene therapy is used for the high replication rate of cytoplasmic replication in target gene expression. Moreover, using anti-sense oligonucleotides (ASO) or using siRNA can be another therapeutic way of targeting the virus. The challenges of this treatment strategy are a lot owing to some compelling reasons. Positive-sense, single-strand RNA (ssRNA) viruses are distinguished by these sequences for optimizing siRNA and preventing through oligonucleotides strategy is pivotal, then the homogeny of this virus's genome should be compared to the conserved sequence of SARS-CoV-2. The entry of oligonucleotide to lung cells should be precisely evaluated (9). These cases are hypothetical in this treatment. Nano lipids can mediate the entry lungs, although this knowledge is also unknown since the amount of siRNA or ASO should be sufficient to control or suppress

infection in the lung. Even if, it is assumed that siRNA is effective clinically. There is a faint possibility for siRNA drug development in the infected population. Therefore, treatment base on siRNA and ASO are used for small infected population and there is no resource for producing rapid drug production (10).

The general route of administration is pivotal for both uptake and expression of RNA. Vaccine administration is classified into two types of injection to reach target tissues such as skin and skeleton muscles called via intradermal (i.d.), intramuscular (i.m), or subcutaneous (s.c.), densely populated by DCs (11). One of the examples of a lipids nanoparticle (LNP) -formulated nucleoside-modified mRNA vaccine was observed in mice after i.m. or i.d. injection during antigen expression. In comparison with i.d. and i.m, administration of LNP-formulated nucleoside-modified mRNA influenza H10 vaccine stimulates protective titers in non-human primates (NHPs) the responses of that were more rapidly after i.d.

There are many of processes which are fundamental for RNA delivery and expression of those vaccines by subcutaneous i.d. and i.m. can contribute to entering to entry mRNA vaccine to target cells such as skin and skeletal muscle accumulated by DCs, and for formulated modified mRNA nucleosides, LNPs are important, and the period of antibody expression was surveyed in mice after i.d. or i.m., both injection (i.d. and i.m.) induced conserved titers H10 in influenza for modified nucleosides mRNA formulated LNP, but they can produce more responses compared to i.d. vaccines (12).

Lipids nanoparticles (LNPs) are designed to use molar ratios of phospholipids that boost fusogenicity and endosomal escape (during this process, mRNA has to relocate in the cytosol to avoid lysosomal degradation), for a novel delivery system to deliver cargo to the cytosol of target cells cationic-ionizable amino lipids should be condensed with nucleic acid at low pH and polyethylene glycol (PEG) lipids which enable steric stabilization of the formulation before employing, and vesicle stability depends on cholesterols not only in vivo but also in vials (2, 13, 14). Furthermore, we have witnessed improving of many amino lipids for siRNA delivery as the US food and drug administration (FDA) in 2018 approved the first LNP-delivered therapeutic siRNA (15). Today, LNP delivery of self-amplifying mRNA and conventional mRNAs are used versus various pathogens like rabies, Zika, and influenza viruses, and they can enhance rapid responses in mice, ferrets, and non-human primates (NHPs). Early and robust induction of IFN-stimulated responses were detected at the site of injection in mice within a few hours. Type I IFNs can stimulate the segregation of prime CD8+ T cells into effector cells. In fact, they inhibit or stimulate the CD8+ T cell response to mRNA vaccines might depend on the intensity of type I IFNs induced (16). One limiting way in the delivery system is releasing the encapsulated genetic material of the endosome into the cytosol. Gilleron et al. (17), reported that less than 2% of LNP-delivered siRNAs are released into the cytosol, and endosomal escape takes place only during a brief phase of endolysosomal maturation. Patel et al. (18) showed that both fusogenicity and late endosome-lysosome formation in the endocytic process is vital during LNP trafficking for releasing mRNA in the cytosol. In other studies, endosomal escape and LNP potency have been discovered by Sabnis et al. (19), who illustrated improvement of delivery routes for new amino lipid and suitable chemistry methods that can design them owing to their efficient fusogenicity and endosomal escape. The difference between in vivo and in vitro data cannot be understood due to using screening of nano vehicles in vitro toward the improvement of mRNA vaccine formulation with enhanced endosomal escape. The dearth or presence of potential interference inactivation of target-specific immune responses through co-delivered mRNA vaccines which are encoding antigens from multiple pathogens has to be evaluated. Brito et al. (2), has improved the delivery of single-vial like LNP and a two-vial method in manufacturing and stockpile is separated from the target mRNA. LNPs are formulated by molar ratios of phospholipids that can bolster fusogenicity, endosomal escape, cationic-ionizable amino lipids which condense with nucleic acid at low pH and PEG lipids to maintain three-dimension formulation and stability (20).

Cationic nanoemulsion (CNE-delivered) self-amplifying mRNA can emerge potent immune responses in mice, rats, rabbits, and NHPs with various viral, bacterial, and parasites infection after two or three immunization, so these ways let the delivery vehicles to be stockpile with the mixture of target RNA and they are designed and produced to be rendered in the outbreak of diseases (21).

Delivered CNE self-replicating mRNA elicited cellular immune response after 2 or 3 injections in mice, rabbits, NLPHs, parasites antigen, and some viral bacteria (22).

As mentioned, mRNA is rapidly degraded by ubiquitous extracellular ribonucleases before being absorbed by cells. For better cellular uptake and efficient mRNA translation in the cytoplasm, mRNA must be combined with polymers or lipids. The choice of the adjuvant is important since it can elicit strong humoral and cell-mediated immunity vital for protection against some pathogens.

It was later shown that the combination of mRNA with lipid is 100 to 1000 times more efficient than DEAE-dextran. The discovery halted the progress of polymeric carriers and paved the way for lipid-based transfection reagents to transport nucleic acids, including mRNA. A comprehensive study comparing poly beta-amino esters (PBAE) and polyethylene (23) polymers with commercial lipofectamine 2000 transfection reagent and propane 1,2-dioleyl-3-trimethylammonium (DOTAP) /1,2-dioleoyl-sn glycerin 3. Phosphoethanolamine (DOPE) was shown to stimulate T cell responses. The first successful test for safety and effective mRNA vaccine using a cationic polymer with intrauterine administration of conjugated PEI- 2KDa to cyclodextrin was obtained, which improved the transfection. Polymeric nanoparticles consisting of biodegradable polymers, such as poly lactic acid-glycolic acid (PLGA), combine well with positive charge hydrophobic molecules. They provide a good colloidal stability, low toxicity, and the possibility of a stable release. Despite the advantages of polymer-based delivery systems, they are not clinically advanced compared to lipid-based delivery systems (24). Lipid-based vectors or lipid-like compounds (lipidoids) are the most common carriers of non-

viral genes(14). To the formation of liposomes derived from various synthetics and natural lipids (complexes of liposomes and nucleic acids are called lipoplexes) or lipid nanoparticles (LNPs), both of them effectively transmit and deliver mRNA-based vaccines. LNPs cationic lipids, made up of triple or quaternary amines, are used for multipolar mRNA formulations. The first use of LNPs was reported as the mRNA delivery system in 2015, which the delivery system consisted of ionized cationic lipid/phosphatidylcholine/cholesterol/PEG-lipid ratio (50: 10: 38.5: 1.5 mol/ mol). The mechanism of mRNA delivery by LNP is not fully understood so far, but it is recommended that LNPs should be induced into the cell by endocytosis and attached to the cell membrane as electrostatic binding. In this system, mediator RNA connects to mRNA Transthyretin-TTR and leads to deposing TTR in amyloidosis patients with inheritance dependent on TTR. This novel drug base on RNAi is called Patisiran with commercial name ONPATTRO™ that recently has been licensed by USA and Europe health organization for intravenous injection (25).

Cytotoxic T cell (CTL) responses against encapsulated mRNA with liposomes after s.c. or intravenous (i.v.) administration measured. Potent CD8+ responses elicited by Lipoplex observed (26). Combined lipid-polymer nanoparticles (LPNs) have previously been shown to deliver effective siRNA in vitro and effective siRNA therapeutic transfer in vitro and in vivo. This hybrid delivery system has shown promising results for mRNA delivery. Recently, a combination platform of polymers and peptides for mRNA delivery has been reported (27). They contain naked RNA, an optimized mRNA sequence, and non-changed RNA. They induce TLR7 with the cooperation of protamine as a cationic protein in the non-coding RNA complex. Also, mRNA vaccines need a delivery route for reaching the target site due to its naked structure degrades by nuclease. Multiple delivery routes are studying contemporary and some encapsulated mRNA molecules connect to positive charge RNA polymers using in charging interactions. The multitude of efforts have been done to produce some particles like viruses that can protect mRNA from endo 5′-3′ exonuclease damage since in this way the efficacy of mRNA in cells increases, consequently, encoding expression of the protein is suitable. In this process, they probably pass the transmembrane and after catching cells can escape from lysosome by endocytosis, and mRNA releases in the cytosol where translation processes occur (28).

Chahal et al. (29), comprised high amine density molecules with organizing branching around mRNA to form monodisperse spheres structures based on developed dendrimer-based nanoparticle system thereby dendrimer-encapsulated self-amplifying mRNA vaccine caused eliciting CD8+ T cell, and antibody responses which wholly protected versus lethal challenge in mice like Ebola virus, H1N1 influenza, and Zika viruses after a single immunization, with 40 mg as the optimal RNA dosage (19). For formulating of six self-amplifying mRNA replicons into in the same dendrimer based-nanoparticle and producing multiplexed vaccine dendrimer technology plays a paramount role in protecting mice versus lethal Toxoplasma gondii combat (2, 29). However, it is still in the first phase and further studies require to use it for potent single-dose vaccines against multiple pathogens and reduce the number and frequency of vaccinations (30).

Another active region for mRNA delivery for this purpose is DCs targeting (31). Englezou et al. (32), tried to improve the targeting of DCs via s.c. injection. In fact, they optimized lipoplex formation, and cationic lipids to rise the uptake, release into the cytoplasm and translation of self-amplifying mRNA molecule in DCs (33). Optimized lipoplex increased in vitro translation in DCs and elicited pro-inflammatory cytokines and both humoral, and T cellular responses against the self-amplifying mRNA-encoded influenza NP antigen in mice and an adoptive transfer model after s.c. vaccination. Unfortunately, studies in vivo with a target of DCs for synthetic mRNA vaccines against most infectious diseases are still in the early phase. In other words, most research has been done for ex vivo mRNA loading of DCs to produce cell-mediated immunity against either cancer or therapeutic vaccination (34) (reviewed in Benteyn et al. (33), and Gornati et al. (34). Lipoplex contributes to increasing translation in vitro and depletes pre-inflammatory cytokines, meaning that they stimulate humoral and cellular responses against self-replicating mRNA coding influenza NP antigen after s.c. vaccination in mice as a delivery model. However, all of these studies with DCs in ex vivo for synthesis mRNA versus infection are in the first stage (35).

Delivery paths may impact on quality and quantity in some mRNA and induce innate immune or even rise synergic effect on adjuvant. One of the promising ways for non-viral RNA is the nanolipoprotein particles (NLPs) function. Cholesterols can enhance vesicles in vivo in viral conditions. Moreover, multiple amino lipids have been developed in recent decades for transferring siRNA. NPLs enter to transmembrane by endocytosis ways. The endosomal process and releasing encapsulated gene agents to cytosol are a limit step for transferring, and it is one of the vital areas for formulation and its quality (22). Endosomal flee takes place only in a short phase from endosomal mature. Petel et al. (36), worked on impure CRISPR in various lysosome ways and found that lysosome-endosome fusion is essential in the endocytic process during transferring LNPs to releasing mRNA in the cytosol. (37). Indeed, the dense amine molecules are able to organize three dense branches around mRNA in the mesosphere. Did they induce CD4+ T cells derived from antibodies against Ebola after immunization by encapsulated unity dendrimers in self-replicating mRNA vaccines development? These dendrimers are made and formulated by multiple vaccines including six replicons. These vaccines can also protect mice against Toxoplasma gondii. Although this technology needs more optimizing with more RNA, its development is promising. An emulsion base on a wide delivery was used before testing for linking self-replicating mRNA to nanoparticles surface.

In 2013, during the outbreak of influenza H7N9 in china against the HA gene in pDNA form, a kind of mRNA vaccine was made after spreading the H1 gene sequence during 8 days (38). The first immunogenicity study was published about the mRNA coding NP influenza virus in mice. However, in 2012, the first whole conserved versus influenza was demonstrated by the mRNA NP vaccine (39). Petsch et al. (40), proved that i.d.

injection by non-changed mRNA with protamine adjuvant coding influenza antigen in mice, ferrets and swine are immunogenic.

The mRNA vaccines either conventional or self-replicate in LNP and CNE were formulated which can stimulate humoral and cellular responses. Chitosan, polyethyleneimine, and formulation have been distinguished base on the self-replicate mRNA dendrimers HA gene (29, 36, 41). Lutz et al. (42), showed that for the first time with 10 μg injection from formulated LNP, mRNA coding HA suppressed hemagglutinin which was more than the human full proper dose of inactive virus compared to vaccination gives rise to inducing immunization (43). Responses can rise by the second dose which can be stable for 12 months, and only a limited immunologic response will emerge during injection and there are a few alterations in cytokines and inflammatory chemokines. The lipoic complex containing mRNA coding influenza HA gene and immunization was administrated in the form of intravenous injection in mice and increase T cells activation. In this vaccine, formulated mRNA by conventional LNP called N1-methyl-pseudouridine used, and mRNA coded HA gene in H10N8 influenza virus and injection was administrated with i.m. and i.d. They demonstrated that memory B cells will rise in plasma after vaccination (3). Recently, modified LNP-mRNA can induce the immune system against conserved virus epitopes during pandemic influenza. In that test, formulated LNP and nucleosides-modified used in conventional mRNA encoding influenza HA antigen. According to some reports, twenty and three candidate were administered intramuscularly by 100μg per dose of this vaccine and after 43 days immune responses observed 87% of humans. Antibody titers in this test can also use in humans (44).

The potency of synthetic mRNA vaccines may be risen by some factors like formulation design, optimization, as well as a combination with adjuvants or surface molecules to target DCs where is the central role of DCs in antigens and initiate antigen-specific related to adaptive immune responses (45). Previously, the mRNA is synthesized, it should be processed through diverse purification steps at the clinical scale and microbeads in batch formats are used, which are easier to employ at large scale to eliminate reaction components containing free nucleotides, enzymes, residual DNA and truncated RNA fragments for the potency of the final product. The inducer of interferon-dependent translation inhibition has been administrated by reverse-phase FPLC at the laboratory scale. After purification of mRNA, it is replaced into the final storage buffer as well as sterile-filtered for next filling into vials for clinical goals (2, 29). RNA can be degraded by both enzymatic and chemical pathways. For ensuring that buffers do not have to contaminate with RNases, antioxidants, chelators, divalent metal ions as well as free- reactive oxygen radical which lead to increasing the stability of mRNA, they should be tested (2, 46).

In the storing and distribution process, thermal stability is one of the major pitfalls, especially in underdeveloped nations where they do not have suitable infrastructures to keep the cold chain. A lyophilized protamine-complexed mRNA vaccine was demonstrated to maintain its wholly biological activity even after exposing to thermal stress situations for several weeks and up to three years at 5 -25 °C as Jones et al. proved that a Kunjin virus-related self-amplifying mRNA freeze-dried in trehalose for about 1 year and was stable at 4-6 °C (47\). Likewise, oscillating temperatures from +4 °C and +56 °C for 20 cycles did not have influence on protective efficiency single-dose of mRNA vaccines which are resistant to high temperature and can be stockpiled and target plenty of pathogens in different diseases simultaneously and decline the frequency of vaccinations, alleviating healthcare worker burden.

The role of adjuvants in mRNA vaccines

Adjuvants can activate innate immunity since their delivery platforms can act as carriers to associated antigens. They can be classified based on their origin, physicochemical properties, and mechanisms of action. Based on their mechanisms of action, adjuvants can be divided into delivery systems (particulate) and immune potentiates (immunostimulatory). For accelerating of mRNA vaccines process, including SARS-CoV-2, some adjuvants which are popular in commercial vaccine producing companies should be used (7);

(1) Classic aluminum adjuvants, make local pro-inflammatory responses, which induce innate immune cells to the site of injection. It can stimulate Th2 immune response upon injection (49). Alum for some formulation of the vaccine does cannot emerge protective and sustained immune responses due to aluminum-containing adjuvants preferentially stimulate Th2 responses (characterized by antibody generation), and in some pathogens, the Th1 immune response (containing cytotoxic CD8+ T cells) is necessary, as a result, for that vaccine alum is not suitable for using, at least alone (50). The TriMix (mix of three mRNA molecules) is an effective adjuvant encoding CD70, CD40 ligand and a constitutively active form of Toll-like receptor 4. Aluminum adjuvants can boost the immune system by facilitating phagocytosis and decreasing antigen disseminate in the injection area which can stimulate Th2 arm in the immune system in the injection site.

(2), MF59 is a kind of emulsion into oil in water with Tween 80 component, squalene, and sorbitol trioleate, and in the USA and Europe have been added in their vaccines (51). MF59 mechanism produces a short peripheral response in the injection site and in comparison with aluminum salts, MF59 causes a stronger immune response, stimulating both T-cell immune response and antibody production.

(3), In adjuvant system (AS) many developed adjuvants by GlaxoSmithKline (GSK) contain AS01, AS02 AS03, AS04 have been employed that AS01 is a lysozyme adjuvant including 3-O-desacyl-4'-monophosphoryl lipid A (MPL) and saponin QS-21. AS02 is an oil-in-water emulsifier including MPL and QS-21. AS03 is a kind of emulsion oil in water containing alpha-tocopherol, Tween 80, and squalene used in influenza. AS04 is also an aluminum adjuvant and has MPL used in human papillomavirus infection and the hepatitis B virus (52).

Indeed, these are adjuvant which can regulate the immune system, although they should be optimized to be effective. Stimulating mRNA immune is increased by one adjuvant like

new and conventional adjuvant. For self-replication RNA vaccines efficiency and immunization will increase after RNA formulation in nanoemulsion with MF59 adjuvant. Optimized mRNA sequence and modified-nucleosides formulated with NLP that produce a substantial innate immune response and modified monocytes, neutrophils, and DCs to the injection area extremely. Both modified mRNA and adjuvant can increase the efficiency of vaccines which are understandable with state-of-the-art technology of RNA.

Optimizing and Stabilization of mRNA vaccines

Strategies such as sequencing engineering or structure to enhance mRNA stability (increasing half-life) and translation are often effective in increasing protein expression. 3′-5′elements UTR sequences and GC as regulating sequences have a vast impact on stability translation, and increase life-span and mRNA expression, which are necessary for mRNA vaccine progress. Exogenous mRNA needs to cross the lipid membrane and diffuse in the cytoplasm to reach the ribosomes and translate into functional proteins (53).

Target cell and immune effectors assume that support rapid uptake in high numbers and they also minimize loss of bioactive compounds owing to extracellular degradation, which is vital for naked mRNA (54). Intranodal delivery (i.d.) of pharmacologically optimized naked antigen-encoding mRNA has been done by Kreiter et al. (8, 55), which was elicited potent antitumor immunity. Furthermore, Diken et al. (56), reported that lymph node internalized and translated mRNA and it also resident both cross-presenting CD8+ DCs and conventional through micropinocytosis. Another research was confirmed by Thielemans et al. (33), that the potency of this delivery platform and format in additional tumor models (8).

The mRNA in cell-based vaccination is electroporated directly into the cytoplasm of DCs in ex vivo, while direct mRNA vaccination encounters diverse obstacles that need to be overcome this specialized molecule to administrate as target. There are some obstacles such as extracellular barriers, endocytic barriers, and intracellular barriers that influence on endosomal escape and mRNA release into the cytoplasm (54).

The mRNA vaccine also needs a delivery route to reach the target site due to its naked structure is degrades by nuclease. Vaccine stability has brought many pitfalls related to delivery and maintaining them, especially in countries where they suffer from the lack of substructure for storing cold chains. For avoiding excessive extracellular degradation and promoting enhanced uptake by DCs complexation or encapsulation of mRNA is necessary (10).

Non-lipid nanoparticles may easily be coupled to targeting ligands, but they are often confronted with the major problems of efficient mRNA release once into the cell. Indeed, incorporation of destabilizing or pH-sensitive polymers is a choice, although convincing vaccination research has not been reported so far (57).

DOPE (1,2-dioleoyl-sn-glycero-3-phosphoethanolamine) a zwitterionic phospholipid employed in combination with cationic lipids as a helper lipid which is known in order to destabilize endosomal membranes. Those nucleic acid lipoplexes which contain DOTAP (1,2-dioleoyl-3-trimethylammonium propane) and DOPE, the mechanism of flip-flop where the positively charged lipids of the entrapped nano-formulations can interact with anionic lipids present in the cytoplasm-expose endosomal monolayer is essential. Peripheral diffusion of the anionic lipids in the lipoplex generates neutrally charged ion pairs thereby the nucleic acids displaced from these cationic lipids and release into the cytoplasm (58, 59).

Knowing the structure of mRNA caused to mRNA molecules highly optimized not only for stability, for translational efficiency. The pDNA templates can encode all structural elements of functional mRNA except the 5′ cap which influences recognition by the translation initiation factor eIF4E, and sometimes the poly A. The stability of mRNA and protein expression are also improved by rising the length of the poly A tail until approximately 120 adenosines since the formation of a circular structure through poly A can bind proteins from the de-capping process and de-adenylating enzymes. The 3′ extension has to be avoided (unmasked poly A tail), for example, by pDNA linearization with type IIS restriction enzymes (Holtkamp et al. 2006). For therapeutic purposes, encoding the poly A tail in the template vector is useful, meaning that their long tail can be used for yielding products instead of enzymatic two-step enzymatic method. Noteworthy, the lipid composition has a paramount role in the stability of the resulting mRNA-liposome complex (60).

After translation of mRNA, encoded protein will be degraded into peptides by the proteasome in the cytosol, entered into the endoplasmic reticulum (ER) for loading toward MHC class I by the secretory pathway shuttled to the surface of cells for presentation to CD8+ T cells. However, when mRNA vaccines deliver antigens directly into the cytosol, they will encounter a barrier of intracellular proteins, and they do not often enter the MHC class II pathway efficiently (61). For being eligible of mRNA-encoded protein for MHC class II presentation, trafficking signals of endosomal or lysosomal proteins resided in MHC class II process have been fused with encoded antigen like lysosome-associated membrane protein-1 (LAMP-1). Secretion signal, domains of MHC class I at the N and C terminal, and the transmembrane are able to improve both CD4+ and CD8+ T cell responses. Kreiter et al. (8, 56) declared that when peptide or protein vaccines with low innate immunogenicity are employed, adjuvants ranging from alum to toll-like receptor (TLR) can induce 'danger signals' to serve immunostimulation (62).

The encoded protein will be degraded into peptides by the proteasome as dsRNA-recognizing protein kinase R (PKR) lead to releasing signaling cascade would interfere with mRNA translation, their activation should be avoided (2). Modified nucleosides, such as 2-thiouridine, N6-methyladenosines, 5-methylcytidine, or pseudouridine suppress PKR activation although by not forming dsRNA (2-thiouridine, N6-methyladenosine) or declining dsRNA pollutants during in vitro transcription (5-methylcytidine, pseudouridine), and HPLC purification were not able to eliminate PKR activation solely and the best way for boosting translation is nucleoside

modification, sophisticated purification, and sequence optimization via maximizing the rate of GC content, however, they depend on immune activation (10). Unfortunately, optimizing of SARS-CoV-2 based on mRNA vaccines is still unknown.

Ongoing vaccines for severe acute respiratory syndrome coronavirus 2 (SARA-CoV-2)

The start of 2020 coincided with the outbreak of the new emerging SARS-CoV-2 pandemic virus from Wuhan, China, which caused severe respiratory syndrome worldwide. This new coronavirus disease is known as a coronavirus disease-19 (COVID-19). The imminent outbreak of the newly emerging virus has prompted all researchers around the world to work on the pathophysiology of the virus and the etiology of the epidemic and to understand the mechanism of the virus pathogenesis and the immune response to it. SARS-CoV-2 is able to attack the respiratory tract system. All research shows that age, defective immune systems, such as immunocompromised, and affecting to other diseases may increase fatality rate of COVID-19 (63).

Still the best way against virus is proper preventive measures, thus, producing vaccine is promising. Up to October 2020 no vaccine produced although according to the result of diverse corporations, on developing effective vaccines are in the last phases (64).

There are three strategies for vaccine; 1) producing whole virus vaccine to induce a rapid immune response in humans against SARS-CoV-2 (65), 2) producing sub-unit vaccines to induce the immune system for the identification of specific viral sub-units. In case of SARS-CoV-2, such research focuses on spike (S) proteins that facilitate binding of virus to the angiotensin-converting enzyme 2 (ACE2) (66), and 3) development of nucleic acid vaccines either DNA or RNA vaccines (67).They should be tested not only for safety, but also their efficiency since owing to a current burdensome circumstance worldwide our needing for having safe as well as effective vaccine is remarkably high.

Immunopathogenesis of SARS-CoV-2

Resident macrophages, and inflammatory monocytes (IMMs) are classified into two big groups based on their function and phenotyping changes: 1) pro-inflammatory macrophages (M1 or activating classical), 2) lifting inflammation macrophages (M2 or activating alternative). In acute infections, monocytes and macrophages often show only a phenotype from active classical macrophages. Macrophages protect the host cells against the viral infection. On the other hand, they can expand lung hurts by generating nitric acid, IL-6, IL-8, IL-1, TNF, and reactive oxygen species (ROS). Moreover, other macrophages can active alternative ways and they show their anti-inflammatory and wound-healing functions by producing Metalloproteinase -MMP and other growth factors and inflammatory phase cytokine, especially TGB-β (68). Both MCP1 and IL-8 are essential cytokines which facilitate neutrophils penetrate, macrophages, and monocyte immigration during the period of infection. IL-6 will be stable and its damage increases. In the presence of inhibitor antibody, the Fcγ receptor and IL-8 decrease substantially. Macrophages heal wounds and in the final recovery that given rise to secreting IL-10 which increases and accelerates the wound healing process and stable homeostasis. When the responses of wound recovery are precisely controlled, this response and structure of normal tissue is conserved rapidly. The virus increased in deceased patients, lung. Furthermore, some factors increase such as IMM, and serum pro-inflammatory cytokine (IL-1, IL-6 IL-8, CXCL-10, and MCP1). Further studies demonstrated that an antibody response versus spike glycoprotein in SARS and the response of neutralizing antibody anti-S (69) increased in deceased patients faster than recovered patients. Neutralizing antibody (NAb) titers in dead patients are more than recovered patients during a special time. These results can show that T cells can conserve SARS-CoV infection, but the response of specific antigen to the virus in lung hurt that derived from SARS is unknown, thus, these function and anti-S-IgG have to be evaluated since anti-S-IgG is effective not only on macrophages, but monocytes, so this is the a-double-edge sword which can lift inflammation and stable it.

Enlisting the antigens for mRNA vaccines to combat SARS-CoV-2

Spike protein (S protein)

The full-length S proteins probably save the correct forms of proteins and able to provide high epitopes and elicit high immunogenic response (7). Further research have been proved that S protein in the form of nanoparticle formulated with alum adjuvants have been shown to produce high levels of baculovirus-neutralizing antibodies in insects (70). S protein is a promising candidate antigen for the SARS-CoV-2 vaccine design, due to several reasons. One reason is the exposing antigen recognized with the host immune system. Secondly, the interaction of antigen with host cells is very high by binding the angiotensin-converting enzyme 2 (ACE2) receptor which has a high affinity for linking to the recipient (7). Recipient ACE2 in lung cells is highly expressed, thus, it is the first target for virus. That is why it attach to lungs after entering to cells. ACE2 has a main role in entering target cells and consequently pathogenicity. Finally, there are homologous proteins for developing vaccines against SARS and MERS. There are 1273 amino acids in S monomer in SARS-CoV-2 with 140 KDa molecular weight (71). Accumulating S protein into a homo-trimer, usually is similar to transmembrane protein fusion class 1. Protein S includes 2 subunits: S1 contains two domains that are N-terminal domain (NTD) and C-terminal domain (CTD), and receptor-binding domain (RBD) located in CTD. S2 subunit includes basic elements that is necessary for membrane fusion. These elements have one internal peptide for membrane fusion called FP and two repetitive 7-peptides called HR, a foreign proximal named MPER, and a transmembrane domain. Recently, a fused trimer of S protein in SARS-CoV-2 with the RBD domain in a complex with ACE2 has been administrated successfully. So far, some high potential S protein fragments have been employed for using antigen vaccine development and include full-length S protein, RBD domain, subunit S1, NTD,

and FP (44). Spike protein of SARS can modulate the pro-inflammatory cytokines (IL-6, TNF-α), meaning that CD4+ induce Th1 which produces IL-6 as well as TNF, as a result, cellular adaptive immunity will active.

RBD (Receptor-binding domain)

S protein in the RBD area interacts directly with the human ACE2 recipient, as a result, it can induce specific RBD antibody, which can prevent viral invasion effectively. Generally, most RBD subunits in vaccines have used as an antigen. For example, the recombination RBD domain includes neutralizing epitopes with diverse shapes that can stimulate high titers neutralizing antibodies versus SARS. Lan et al. (73), reported that immunized rhesus with RBD formulated with alum adjuvant that can release neutralizing antibody that decreases viral respiratory *infection* caused by the *MERS*-coronavirus (*MERS*-CoV). Nyon et al. (74), reported that transgenic fused hCD26/DPP4 to neutralize antibodies FC with RBD can prevent *MERS*-CoV. Also, this domain is conserved with multiple neutral epitopes.

NTD (N-Terminal Domain)

Similar to RBD, S protein N-terminal domains (NTD) has carbohydrate domain binding receptor activity. For instance, the spike protein NTDs of transmissible gastroenteritis virus (TGEV) attach to sialic acid, therefore, this domain is one of the great candidates for vaccine development. Moreover, regarding one study, the recombinant N-terminal domain (rNTD) of the spike (S) protein of MERS-stimulates cell immune and neutralizing antibodies in mice. Due to the genome of coronavirus is changeable it needs to use various epitopes antibodies to escape from the immune system, although this function has not been defined (76). MERS-CoV S-NTD protein stimulates potent cellular immunity and antigen-specific neutralizing antibodies was protective against the viral challenge in mice.

S1 subunit

As mentioned above, this part contains RBD, and NTD, that are suitable for vaccine development. Wang et al. reported SARS S1 protein is formulated with conserved transgenic hDPP4 adjuvant MF59 in mice against lethal viruses.

FP (Fusion peptide)

FP domain is a subunit of S2 which plays a paramount role in fusing membrane of viruses as it causes virulence, so it is an antigen candidate for preparing vaccines.

Nucleocapsid protein (N protein)

This protein is abundant in coronavirus and its sequence is conserved with 50 KDa molecular weight. This protein has a multitude of fused states including nucleoside kind and budding transduced signals. It replicates from RNA and transcript from mRNA, so the most antigenic part is related to N protein. In 89% of SARS recovered patients, we can see that they produced an antibody against this antigen. In some reports, N protein stimulates CTLs in poultry bronchitis which can reduce clinical symptoms and virus clearance from the lungs. It is recommended that cellular immune is necessary for N protein. In contrast, according to reports, immunization with N protein causes a difference in neutralizing antibody responses, meaning that it was not conserved in hamsters (79). As a result, this can be used as a marker in COVID-19 due to its immunogenicity, whether it can be employed for vaccines or not is challenging (64). DNA vaccine encoding SARS-CoV N protein was able to produce strong N-specific both humoral and cellular immune responses in vaccinated C57BL/6 mice, and it also reduced the titer of the vaccinia virus during the challenge. There are some arguments whether cellular responses to N protein are necessary for vaccine development or not although no one can disregard their significance in diagnostic assays due to its high immunogenicity as a marker (73). N protein can be used for therapeutic mRNA vaccines.

Membrane protein (M protein)

M protein with 25 KDa molecular weight is a glycoprotein transmembrane virus and contains virus elements. M protein is affluent on the SARS surface. Full-length M protein can induce a neutral antibody in SARS patients. The structural and immunologic analysis illustrates the domain of transmembrane M protein contains T cell epitopes and induces strong cellular immune responses. M protein is conserved during the evolution of diverse species, so it is an antigen candidate for vaccine development (35). According to immunogenic and structural analysis, a strong cellular immune response is stimulated by the trans-membrane domain of the M protein includes a T cell epitope cluster.

Envelope protein (E protein)

E protein is small and integral membrane protein contains several features in COVID-19. It plays a paramount role in the virus's life cycle, assembly, budding, envelope formation, and pathogenesis. Although S, N, and M proteins are immunogenic, E protein is not immunogenic since it has only 76-109 amino acids as a channel in the coronavirus, which can decline its immunogenicity. E protein makes virulence factor as in inflammatory factors IL-1β, TNF, and IL-6 will decrease after destroying E protein (35).

Although the linear epitopes of B cell and T cell in SARS-CoV are known as a model, we are not able to find linear epitopes in bioinformatics prediction of human B cell and T cell epitopes in SARS-CoV-2. Indeed, there are five regions including (residues 274-306, 510-586, 587-628, 784-803 and 870-893) on the S glycoprotein of SARS-CoV expected that being associated with a boost immune response, while other research highlighted that candidate epitopes need the validation of the sample of the patient in humans (80). It is obvious that for vaccine design, the viral spike should be targeted from acid nucleic based methods to elicit neutralizing adaptive immunity at the site of viral entry. Viral glycosylation plays an important role in mediating protein folding, stability, and shaping viral tropism (69). SARS-CoV has a high sequence similarity to SARS-CoV-2 in terms of epitope response.

According to bioinformatics predictions, there are B cell and T cell epitopes in SARS-CoV-2 which are promising targets for immune recognition as well as vaccine design (81). E protein may not be glycosylated on N66 to improve the functioning of E

in other capacities vaccinated models in animals developed boost immune responses (cellular and humoral) and were protected versus infective challenges. Neutralizing antibodies require to act versus spike proteins and Nucleocapsid (82). N protein can produce CD8+ T cells in coronavirus (23). CD4+ T cell response to spike protein, the major target for vaccine efforts associated with the magnitude of the anti-SARS-CoV-2 IgG and IgA titers. In other words, the S protein includes significant virus-neutralizing epitopes which elicit neutralizing antibody in the host species (83). N protein is internal in SARS-CoV, and it is more conserved than surface proteins like S and M, therefore, it can be considered for the protective vaccine as it induces cellular immune response (84). M protein is conserved glycosylation, and similarity of 3a protein to them contributes to finding the glycosylation of these two SARS-CoV membrane proteins so it is used for virus T7 expression system separately.

Mechanism of the immune response induced by mRNA vaccines (Fig. 2.)

Efforts for vaccine design versus SARS-CoV-2 is continuing. Impressive vaccination could have a paramount role in inhibiting viral infections in the human population. The high similarity between SARS-CoV-2 and SARS-CoV according to immunological studies may prove that epitopes B cell, T cells that originated from spike S1 RBD protein and N (Nucleocapsid) and they are alike with SARS-CoV-2 protein and they are considered as a new purpose in design vaccines, especially for prophylactic vaccines. Epitopes are also great for new antiviral goals and so far, we have not seen any mutations. The dearth of immunological knowledge about this novel virus-like immunologic epitopes which can release T cell antibodies (47).

Genome phylogenetic analysis entry to cell mechanism, using recipient on human cells, surface, and understanding conserved immune responses and its similarity with COVID-19 probably help us in expansion and preparing vaccines against COVID-19. Several reports about SARS-CoV highlight the role of the humoral and cell-mediated adaptive immune response. The most antibodies responses versus S protein in infection observed in mice. Moreover, high protein expression against N protein in SARS has the most immunological effect during the infection. Although it stimulates short antibody response in patients infected by SARS-CoV, after infection even 10 years it makes long-term T cell responses. Therefore, those are a candidate for future vaccine design. The structural proteins of SARS-CoV-2 have much higher immunogenicity for T cell responses than the non-structural proteins, so they should be considered carefully.

Phylogenic analysis of structural proteins illustrates M, S, and N proteins of SARS-CoV-2, and SARS-CoV has similar genetics while they have an obvious difference in S protein (86). On the other hand, there is a low similarity in all proteins of SARS-CoV-2, and MERS-CoV. Therefore, research about SARS-CoV-2 immunological structure proteins can be used for vaccine development. According to one statistic research about SARS-CoV, there are B cells epitopes including antigenic peptides and discontinuous epitopes contain conformational epitopes in SARS-CoV-2 that plenty of them derived from S and N proteins and a few of them derived from M. Linear and discontinuous epitopes of S protein proved that this protein has two functional subunits: subunit S1 for interacting of the virus with host cells or receptor and subunit S2 that is fused with membranes and cellular. As a result, antibodies targeting linear epitopes in subunit S2 may be important in neutralizing and interaction against SARS-CoV-2. However, subunit S2 is less exposed than S1, so it is probable they can be recognized by antibodies during a conformational alteration in the process of viral entry coronaviruses. It is not obvious that weather SARS epitopes can be effective for COVID-19 or not. Unfortunately, there are not any effective drugs versus SARS-CoV-2 (87).

Immune potentiators can activate innate immune responses through pattern-recognition receptors (PRRs) or directly such as cytokines. PRRs have diverse classes of receptors (Toll-like receptors (TLRs)), nucleotide-binding oligomerization domain (88) like receptors (NLRs), and the retinoic acid-inducible gene-I-(RIG-I-) like receptors (RLRs) which are extensively expressed on immune cells. Their engagement by pathogen-associated molecular patterns (PAMPs) causes the activation of those innate cells which can be matured or migrate to other tissues and generate cytokines as well as chemokines.

mRNA vaccines safety

We can observe most patients (80%) with COVID-19 infection experienced a mild illness that approximately 14% experienced severe disease and 5% were critically ill (53).

Consequently, COVID-19 presents acute respiratory syndrome, however lymphocytes T-lymphopenia high circulating rate of pro-inflammatory cytokines and chemokines, accumulating a group of neutrophils and macrophages in lung and immunosuppression. Increasing cytokines and pro-inflammatory chemokines in blood and accumulation of neutrophils and macrophages in lungs disrupt immune system regulation, meaning that active non-neutralizing antibodies against diverse S domain in an alternative pathway by Fc recipient raising damages result from COVID-19 damages. Regarding various studies, we can see that the vaccination of volunteers stimulates antibody-dependent enhancement (ADE) contains phagocytic antigen-presenting cells (APC) effect on T cells have an important function in controlling coronavirus infection (53).

Pan-T depletion is present in sever COVID-19 infection and may accelerate ADE risk. Surveying sequences and S motif conserved structure illustrates MERS-CoV and SARS-CoV vaccines can bring ADE, which overshadows COVID-19. The ADE infection pathway grants SARS-CoV an opportunity to infect primary human macrophages, but it does not sustain productive viral replication in the infected cells (10).

Autophagy inhibitors cause a decrease in APC infection and T cells. Polyproteins ORF1a and ORF1ab are divided into 16 non-structure proteins from nsp1 to nsp14. In the early stage of this disease, inhibition of innate immune may inhibit SARS-CoV-2, but in the second phase it will become more severe and in the second phase improvement of disease starts with adaptive humoral antibodies (one to two weeks after infection) contemporary (90). MERS can infect macrophages derived from

monocytes and dendritic derived from monocytes and T cells, however, the infectivity of SARS-CoV-2 in cellular population either with non-neutralizing antibody or without non-neutralizing antibody is still unknown. Released chemokines from infected cells are easily able to attract free immature macrophages and dendritic cells in potent people; therefore, it can reinforce immune cell disruption. In some patients with severe infection, this issue active overly macrophages which can bring chemokines and cytokines. In some cases, SARS-CoV-infected patients the rate of CD4+ and CD 8+ T cell decline (53). Infection derived from macrophages and some T cells with disrupting in virus cellular paths regulation can contribute to decreasing innate immune as well as humoral in patients during a second phase and increase infection severity. The genetic difference in MHC class 1 and B1 receptor in INL 12 and their improvement have been recognized (53). Epigenetic changes can block MHC class in viral respiratory infection caused by the MERS-CoV and increase T killer cells as well as some T cells lead to death derived from MERS-CoV (53). Other differences in diseases may cause disparate cell populations with host receptor (ACE2) for SARS-CoV and COVID-19 that ACE2 expresses severely in the lungs. Neutral mutants may provide antigenic drift for viruses to escape the immune system. Some small drug molecules or prophylaxes have a great chance against SARS-CoV and COVID-19. As a matter of rule, accessory proteins and non-exposed replicas have plenty of conserved long peptides targets to select continuous fragments of critical residue for T-cell epitopes vaccines (91).

Today, we face umpteen insolvable questions about this novel virus especially about humoral as well as cellular immune responses, which are indispensable for vaccine design. Nowadays, in most countries, institutes of research development (R & D) shifting their research and development (R & D) effort to focus on COVID-19 vaccine improvement. After vaccines design and provision, it is pivotal to evaluate their safety, quality, and efficiency before clinical phases. Generally, three clinical phases are required for that reason. The development of new vaccines may take a long time approximately 10 to 20 years. Less than 10% of clinical phases were successful and FDA (Food and Drug Administration organization declared between 3000 candidate vaccines in clinical phases less than 20% conformed and entered to markets from this organization for 30 years. Regarding geographical publication, there are two various forms for COVID-19 called S and L (92). Spread ability and severity of COVID-19 bring challenges to vaccine design (36). Knowing immunology in prevention modern RNA base vaccines is precise owing to those are for healthy people. Indeed, working with the mRNA does not require any chemical toxic materials or cell cultures, meaning that manufacturing mRNA does not have difficulties associated with other vaccines systems such as live viruses, viral vectors, inactive vaccines, and subunit protein vaccines. Furthermore, producing mRNA needs a little time, and vaccinated candidate with mRNA, will not face a dangerous risk with infections and does not integrate into the host genome, so that is safe. Safety is very important for designing vaccines and drugs whereas many researchers conformed that preparing vaccines versus SARS-CoV-2 without safety can be disastrous. For example, S protein is one of the candidates for vaccine development, but other biologic interactions can be affected such as linker domain receptor, RBD, and fusion membrane. According to last research full-length genome of S protein can hurt liver severity, as a result, it can increase infection because of stimulating ADE (antibody-dependent enhancement) derived from specific antigens and increasing virulence by coronavirus causes hepatitis in murine probably due to one glycine amino acid in 310 amino acids residues from spike glycoprotein and mutation in this area leads to hurting CNS (Central nervous system) while it is unknown that domains and amino acids in S protein can hurt to the liver, thus, full-length S protein disrupts immune system when it acts as an antigen (93). For solving this problem most studies focused on the structure and function of that protein and many mutants are considered in antigen design whereas one clinical trial in vaccine development was done for evaluating immune and immunogenicity of developed 1273 mRNA and they are evaluated by the institute of evaluating and development vaccines.

Clinical trials in mRNA vaccines

Animal models are vital for vaccine efficiency before clinical analysis. Recently, one study has been done about the role of transgenic h2CE2 in infected mice by COVID-19 and evaluated virus pathogenicity. Moreover, Virus-like particles (VLPs) are multiple molecules that imitate defined native viruses, although it has no genome. They are safe and economical using during infections. Take VLPs, which can stimulate neutralizing antibodies and have been used in H5N1 and SARS vaccines as an example. In the absence of animal models, using VLPs is an alternative path that can be evaluated the effectiveness of COVID-19 vaccines (94). In comparison with cancer prevention and treatment ways, a few clinical studies have been done for mRNA vaccines. Clinical evaluation using dendritic transfected cells with mRNA encoding diverse antigen HIV 1, pp65, and cellular molecules in CMV (cytomegalovirus) showed mRNA vaccines are safe. They stimulate cellular immune responses associated with CD+4 T and CD+8 T cells derived from antigens. The recent result of mRNA vaccines with protamine vaccine against rabies showed RNA with protamine is safe too and it is tolerated dose is substantial, although that efficiency depends on the dose and administration method. For this reason, needle-free injections are used instead of injection with non-needle. The phase I clinical trial (NCT03076385), shows that formulated mRNA vaccines with LNP against H10N8 lead to inducing an effective humoral immune response in 23 volunteers with mild symptoms (5, 51). Metastatic melanoma with the route of intranodal administered is in phase I/II trial (NCT01684241), and demonstrated an increase in tumor-specific T cell responses in some patients (5, 27).

Good manufacturing practices (GMP) production has a correct strategy according to recommended guidelines by trading food, beverages, and cosmetics medical products, dietary supplement, and medical devices agencies to evaluate the quality of products. Preventing damage is the main goal of GMP. Although the demand for vaccine development is high and many medical companies invest in it, they should regard to GMP. For this issue, some implements such as increasing the risk of infection,

personnel safety, cleaning rooms, and equipment related to producing vaccines should be considered.

Future prospective of mRNA vaccines

Routinely, at least 12 to 18 months is needed to develop a new vaccine (95). Owing to the quick spreading of COVID-19, mRNA-based platforms offer great flexibility in terms of antigen manipulation to prevent a second wave of this pandemic (96). On March 2020, Moderna launched clinical testing of its mRNA-based vaccine mRNA-1273 against COVID-19 just 2 months after sequencing the virus. The mRNA-1273 express for a stable form of the SARS-CoV-2 spike protein and formulated into an injectable form using a novel lipid nanoparticle (LNP)-liquid nano particle (97).

Even with hopes of an mRNA-based vaccine targeting the S protein, it is essential to note that the SARS-CoV-2 uptake to host cells via antibodies through the antibody-dependent enhancement (ADE) mechanism. The ADE can stimulate sustained inflammation, lymphopenia, and/or cytokine storm(98). Homology modeling revealed that SARS-CoV-2 had a similar RBD structure to that of SARS-CoV, in spite of amino acid variation at certain key residues (99). Li Liu et al. (24) showed that the presence of anti-S-IgG in SARS-CoV both can hurt lungs at the first stage of infection and result in the lack of wound-healing macrophages. In addition, the interaction between S protein and the immune response happens in numerous ways. The ratio of outer surface S protein per SARS-CoV-2 particles (s/v) possibly will predetermine whether the IgG able to efficiently neutralizes the virus when there are redundant S proteins and how many vaccine boosters are required to effectively neutralize the virus without concerns about the existence of redundant S protein complexes that may unblocked S protein available for processing and binding to ACE2. This remains unclear for SARS-CoV-2, and it is a challenging factor to develop vaccine against SARS-CoV-2.An vital consideration regarding to mutations in the S protein that may affect the degree of viral binding affinity to host cell ACEII receptor (100).Therefore, it is challenging to certify that the new vaccine that is targeted to S protein can be administrated long term.

The high glycosylation pattern of envelope proteins in SARS-CoV-2 works similar invisible camouflage, which can aid the virus to efficaciously escape the immune system (101). Consequently, the higher the degree of glycosylation, the easier the chance that the virus will overcome the immune response and the lesser the success rate of vaccine manufacturing. So, circumventing the highly glycosylated viral envelope is another pitfall of SARS-CoV-2 which leads to development of a vaccine extremely difficult especially in traditional whole virus particles vaccines. Although, the targeting only the immunodominant domain in the surface protein of SARS-CoV-2 based on mRNA-based vaccine technology instead of entire virus particle, may lead to the human immune system raising neutralizing antibodies without the impact of viral glycosylation. It is recommended that the bacteria can be used to produce mRNA vaccines and provide large-scale mRNA instead of in vitro transcription.

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