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REVIEW ARTICLE

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Mechanism of modified mRNA structure in COVID-19 vaccines for inducing neutralizing antibodies

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ABSTRACT

The development of SARS-CoV-2 mRNA vaccines is closely linked to advancements in mRNA manufacturing technology. Structural modifications, such as replacing uridine with 1-methylpseudouridine (1mψ), enhance translation efficiency and help the mRNA evade immune detection. Lipid nanoparticles (LNPs) serve as an effective delivery system. Vaccines like BNT162b2 and mRNA-1273 target the receptor-binding domain (RBD) of the spike (S) protein, prompting B cells to produce neutralizing antibodies that block the RBD from binding to the Angiotensin-Converting Enzyme 2 (ACE2) receptor, preventing infection. These vaccines also stimulate adaptive immune responses by activating CD4+ and CD8+ T cells, with mRNA functioning as an endogenous antigen. Antigen-presenting cells (APCs) present the vaccine antigens via major histocompatibility complex (MHC) class I and II pathways, with CD8+ T cells recognizing MHC class I and destroying infected cells, while CD4+ T cells recognize MHC class II and assist in B cell maturation and antibody production. While mRNA vaccines have proven effective in neutralizing SARS-CoV-2, challenges remain, including the decline in neutralizing antibody titers over time and the emergence of new viral variants.

Keywords: mRNA vaccine, neutralizing antibody, SARS-CoV-2

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the virus responsible for coronavirus disease 2019 (COVID-19), was first identified in Wuhan, China, in December 2019 [1]. COVID-19 primarily affects the respiratory system and spreads via respiratory droplets, leading to its rapid global dissemination. As of August 19, 2022, the World Health Organization (WHO) reported 591.7 million confirmed COVID-19 cases and 6.4 million deaths worldwide [2]. In Indonesia, 6.3 million cases and 157,300 deaths were recorded [3]. The spread of the virus has been worsened by mutations and the emergence of new variants, such as Omicron BA.4 and BA.5, first detected in Indonesia on June 6, 2022 [4].

Vaccination has been the primary global strategy for mitigating the COVID-19 pandemic [5]. Current vaccine platforms include live-attenuated, inactivated, protein subunit, viral vector, and nucleic acid vaccines [6]. Among these, mRNA vaccines, a type of nucleic acid vaccine, have shown particular effectiveness. After two doses of the mRNA vaccine (BNT162b2), COVID-19 cases caused by the alpha and delta variants were reduced by 7% to 23% [7]. Phase III clinical trials for mRNA vaccines like mRNA-1273 and BNT162b2 have shown exceptional efficacy, providing 94.5% and 95% protection, respectively [8,9]. These vaccines were the first to receive emergency use authorization from both the U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA).

The success of mRNA vaccines in preventing SARS-CoV-2 infection is largely due to advances in mRNA technology and the vaccines' ability to trigger a robust adaptive immune response. While mRNA vaccines for other diseases like Zika, HIV-1, and influenza are still under development, COVID-19 mRNA vaccines were the first approved for emergency use in a pandemic setting [9]. The development of mRNA COVID-19 vaccines has focused on stimulating the production of neutralizing antibodies and T-cell-mediated immunity against SARS-CoV-2 [10]. Since the viral spike protein plays a critical role in SARS-CoV-2's binding to the ACE2 receptor, understanding how mRNA vaccines induce neutralizing antibody production is crucial. This paper will examine the molecular modifications in COVID-19 mRNA vaccines and their mechanisms for generating an adaptive immune response, particularly focusing on the production of neutralizing antibodies.

Methods

This narrative review was conducted through a comprehensive literature search of major academic databases and reputable sources. We conducted searches in PubMed, Scopus, and Google Scholar, and reviewed official documents from government agencies and international organizations. The primary search terms included "SARS-CoV-2 infection," "mRNA vaccine," "immune response," and "neutralizing antibody," with Boolean operators used to optimize the search strategy. Articles were selected based on their relevance to these key topics, with priority given to peer-reviewed studies and official reports. The screening process involved an initial abstract review to assess relevance, followed by an in-depth analysis of full-text articles that met the inclusion criteria.

The mRNA COVID-19 vaccine

The development of mRNA vaccines marks a major breakthrough in molecular biology, building on decades of research. In the 1980s, scientists first successfully delivered mRNA into cell lines using liposomes, a milestone that laid the groundwork for mRNA vaccine technology. The success of these vaccines is the result of advancements across multiple fields, including molecular biology, lipid chemistry, pharmacology, immunology, and virology. Chemists and pharmaceutical scientists played a key role in developing liposomes, which enabled the efficient delivery of mRNA into cells. Immunologists contributed by identifying the pseudo-uridine modification, which reduces mRNA's immunogenicity, while virologists were instrumental in sequencing the SARS-CoV-2 genome. Molecular biologists further demonstrated the in vitro expression of proteins through mRNA transferase [11,12].

COVID-19 vaccine platforms can be categorized into conventional and non-conventional approaches. Conventional vaccines include live-attenuated and inactivated virus vaccines, while non-conventional platforms encompass protein subunit vaccines, replication-deficient viral vectors, and nucleic acid vaccines (both DNA and mRNA) [6]. Among these, mRNA vaccines—such as mRNA-1273 and BNT162b2—have shown high efficacy, with 94.1% and 95% effectiveness, respectively, in preventing COVID-19 [8,9]. These vaccines were the first to receive emergency use authorization from the FDA and were also approved by the European Medicines Agency (EMA) during the pandemic. However, challenges remain, particularly regarding their storage and delivery, as mRNA vaccines must be kept at ultra-low temperatures to maintain stability. The use of lipid nanoparticles (LNPs) for encapsulation is crucial for stabilizing and delivering the mRNA [8,9].

The core of mRNA vaccine technology involves introducing pathogenic messenger ribonucleic acid (mRNA) into the body, where it is translated into target proteins by cells. This mimics a natural infection and induces an immune response similar to that triggered by conventional vaccines [11]. Unlike traditional vaccines that deliver protein or inactivated pathogens, mRNA vaccines use nucleic acids, which need to enter cells to be translated into antigenic proteins. However, naked RNA is highly susceptible to degradation by RNAse enzymes outside cells. The development of liposome-based delivery systems

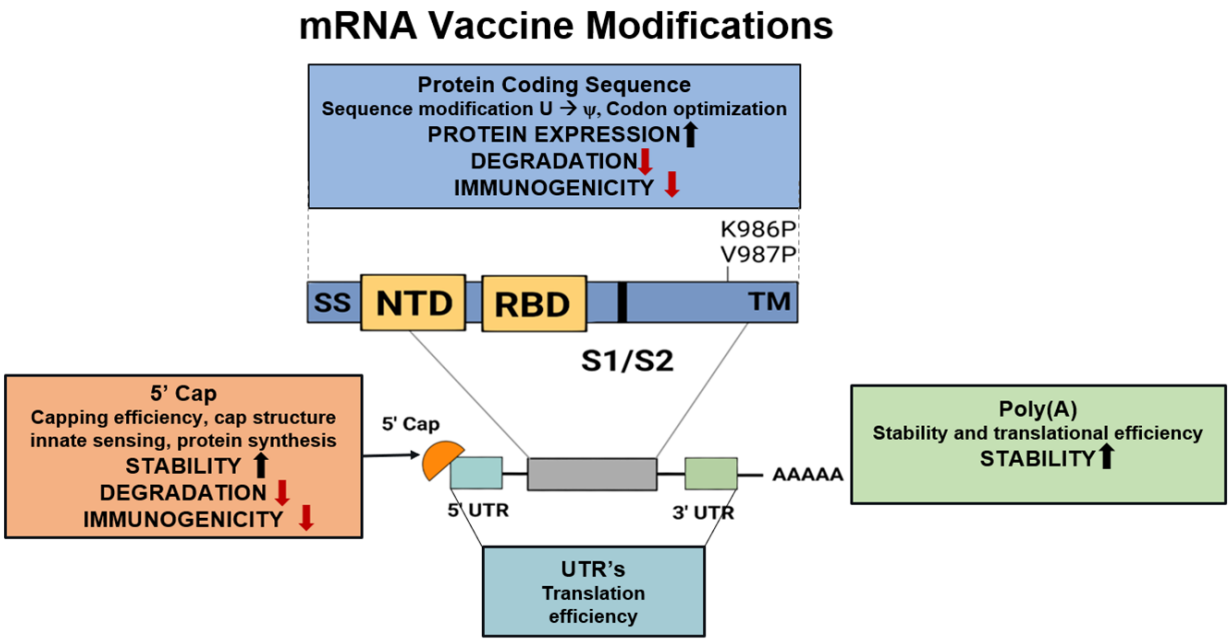

Figure 1. mRNA vaccine modifications in COVID-19 [14]

addressed this issue by encapsulating the mRNA in lipid vesicles, which protect it from degradation and enhance cellular delivery [13].

Additionally, the human immune system is particularly sensitive to foreign RNA, detecting it via Toll-like receptors (TLRs). Activation of these receptors by foreign nucleic acids can inhibit protein antigen synthesis and degrade RNA, which impedes the formation of adaptive immunity. Immunologists discovered that incorporating pseudo-uridine into mRNA reduces its immunogenicity and increases its stability. This modification helps mRNA evade detection by TLRs, thereby promoting a successful adaptive immune response [12].

The molecular structure of mRNA vaccines

mRNA is a single-stranded nucleic acid that carries genetic information, later translated into proteins. Its structure consists of an open reading frame (ORF), untranslated regions (UTRs) at the 5' and 3' ends, a 7-methylguanosine cap at the 5' end, and a poly-A tail at the 3' end (Figure 1). In developing therapeutic systems such as mRNA vaccines, modifications to these structural components enhance stability, prevent degradation by RNases, improve translational efficiency, and stimulate immune responses [14]. These modifications are essential because RNA is inherently unstable and prone to degradation via hydrolysis, nucleases, oxidation, and chemical reactions [15].

The ORF contains the protein-coding sequence, where codon optimization is crucial for enhancing protein translation and proper folding. By modifying the ORF, protein production rates and ribosomal stability can be improved. Codon optimization varies between organisms; for example, prokaryotes and eukaryotes require different codon adaptations. Aligning the nucleic acid sequence with the target amino acids is essential for maximizing protein expression [16]. Additionally, the GC content of mRNA plays a role in stability, as excessive GC content can disrupt secondary structures and affect protein folding [14].

Modifications to the uridine nucleotide have significantly increased mRNA stability within cells. Uridine can be altered to pseudouridine (ψ) or N1-methyl-pseudouridine (1mψ), which improve secondary structure and increase translational efficiency by up to 1.5 times in HeLa cells [17]. These modifications occur naturally in various RNA species, including tRNA, rRNA, snRNA, and mRNA. Pseudouridine strengthens hydrogen bonding, leading to greater stability compared to unmodified uridine [18].

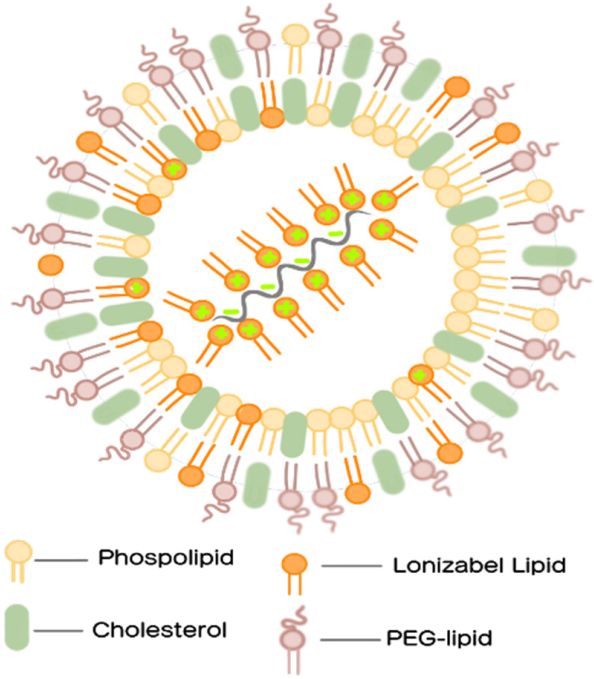

Figure 2. mRNA-lipid nanoparticle (LNP) delivery system [24]

Unmodified mRNA, which contains the uridine base, is highly immunogenic and is recognized by Toll-like receptors (TLRs), particularly TLR 3, 7, and 8, which trigger type I interferon (IFN) secretion. This immune activation enhances dendritic cell activity and triggers a strong T cell response [19,20]. However, excessive immunogenicity can hinder adaptive immunity by causing premature mRNA degradation. Studies have shown that modifying mRNA with pseudouridine reduces immune activation and enhances protein expression. For example, in animal models, mRNA modified with 25% thio-uridine and 25% 5-methyl-cytidine resulted in higher protein expression compared to unmodified mRNA [13].

Further improvements are achieved with the modification of mRNA using 1mψ, which adds a methyl group to the N1 position of pseudouridine. This modification increases mRNA stability and reduces cytotoxicity compared to pseudouridine alone [19]. Naturally found in 18S rRNA and tRNA, 1mψ allows mRNA to evade immune detection, facilitating efficient translation without premature degradation. Studies using lipid nanoparticle-delivered mRNA with 1mψ have shown protective antibody production against HIV-1 in animal models [13].

The 5' and 3' UTRs also play a vital role in regulating mRNA stability and translation. Their interaction with RNA-binding proteins can affect mRNA degradation rates. Optimizing these regions often involves minimizing secondary structures and shortening the UTRs, as shorter 5' UTRs have been shown to improve translation efficiency [21,22]. The 5' cap, composed of 7-methylguanosine (m7GpppN), is essential for protecting mRNA from exonucleases and enabling translation initiation. This cap structure mimics natural eukaryotic mRNA, helping the mRNA evade immune detection [14].

The poly-A tail at the 3' end is crucial for regulating mRNA stability and initiating translation. In mammalian cells, poly-A tails typically contain about 250 nucleotides, gradually shortening over time. For synthetic mRNA, a poly-A tail length of around 100 nucleotides is optimal for modulating degradation and maintaining stability [14].

The mRNA vaccine delivery system

The delivery system for mRNA vaccines is a critical component of their development and success. For COVID-19 mRNA vaccines, lipid nanoparticles (LNPs) are employed to protect the mRNA from degradation by endonucleases as it circulates in the body and is delivered into cells. Once inside the cell, the mRNA is released into the cytoplasm, where it can initiate protein translation. LNPs, the primary delivery vehicle for these vaccines, consist of four main components: neutral phospholipids, cholesterol, polyethylene glycol (PEG) lipids, and ionizable cationic lipids (Figure 2) [23,24].

The LNPs encapsulate the mRNA by using positively charged amine groups at low pH, which interact with the negatively charged mRNA during the manufacturing process. This positive charge not only aids in mRNA encapsulation but also facilitates the fusion of LNPs with the cell membrane during internalization. PEG lipids play a crucial role in stabilizing the nanoparticles, controlling particle size, and preventing aggregation during storage. The rapid mixing technique used in production results in particles within the size range of 60-100 nm [25].

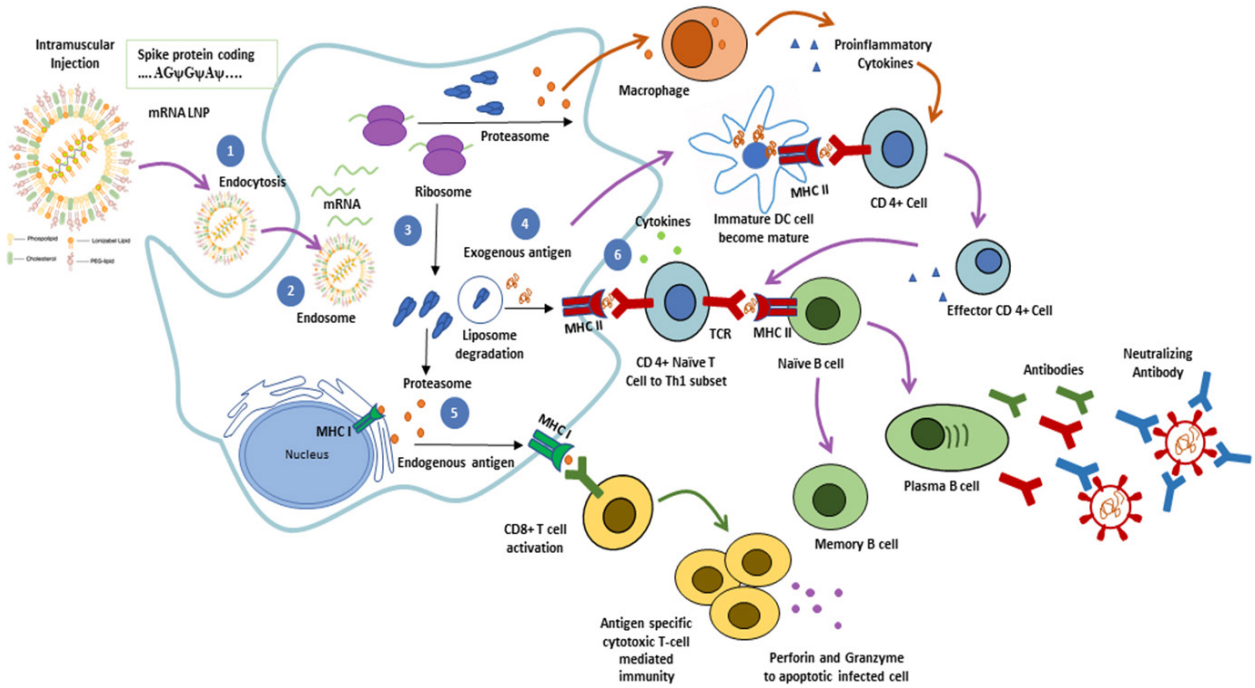

Figure 3. The mRNA vaccine mechanism to stimulate adaptive immune responses [28]

Additionally, LNPs contain apolipoprotein E (ApoE), which is essential for the internalization of mRNA via endosomal pathways, further protecting the mRNA from degradation. Although the precise mechanisms by which LNPs safeguard mRNA are still not fully understood, it is known that the ionizable lipids in LNPs become protonated in the acidic environment of the endosome (pH <7). This protonation promotes lipid exchange between LNPs and the anionic phospholipids in the endosomal membrane, leading to membrane fusion. This fusion enables the release of mRNA into the cytosol, where it can be translated into proteins. ApoE enhances this process by helping the mRNA evade the endosomal degradation pathway [26].

Mechanism of mRNA vaccine in stimulating neutralizing antibodies

One of the key indicators of a successful vaccine is its ability to stimulate a strong adaptive immune response, involving both B cell and T cell activation. This response is essential for long-term protection against pathogens [27]. The development of SARS-CoV-2 mRNA vaccines has focused on efficiently inducing the production of neutralizing antibodies, which block the virus from entering host cells and prevent severe infection. In addition to neutralizing antibodies, CD8+ and CD4+ T cell responses are crucial for fighting SARS-CoV-2. Memory T cells expedite viral clearance by killing infected cells and enhancing antibody production. Because mRNA vaccines deliver endogenous antigens, they more effectively stimulate both CD4+ and CD8+ T cells, mimicking natural infection more closely than vaccines using exogenous antigens like protein subunits or inactivated viruses [27].

After intramuscular administration, the mRNA vaccine is taken up by muscle cells through endocytosis. The lipid nanoparticles (LNPs) provoke a local inflammatory response, recruiting immune cells such as neutrophils, monocytes, and dendritic cells (DCs). Once the mRNA is released into the cytosol, it is translated by ribosomes to produce the spike (S) protein. This foreign protein is then degraded into smaller fragments, which are presented as antigens to immune cells, such as macrophages and DCs. Upon recognizing the antigens through pattern recognition receptors (PRRs), macrophages phagocytose the antigens and secrete pro-inflammatory cytokines, initiating the adaptive immune response [28].

Antigen-presenting cells (APCs), such as DCs, present the spike protein to CD4+ T cells via MHC class II molecules. This activation triggers the release of cytokines, which stimulate naive B cells to differentiate into plasma cells, producing specific antibodies. Simultaneously, DCs and other nucleated cells present antigens via MHC class I molecules to activate CD8+ T cells, which release perforins and granzymes to destroy infected cells, further enhancing the immune response (Figure 3) [28].

The neutralizing antibodies produced by mRNA vaccines are expected to provide long-term protection. Following antigen exposure, CD4+ T cells stimulate B cells in secondary lymphoid organs, leading to affinity maturation in germinal centers (GCs). During this process, B cells undergo somatic hypermutation, increasing their affinity for the antigen. These high-affinity B cells differentiate into long-lived plasma cells (LLPCs) and memory B cells (MBCs) [29]. LLPCs continue to secrete neutralizing antibodies, maintaining immunity by circulating in the blood and responding immediately upon re-exposure to SARS-CoV-2. MBCs rapidly proliferate and differentiate into plasma cells when re-stimulated by the antigen, significantly boosting antibody levels upon re-exposure [30].

DCs, especially, play a vital role in presenting the spike antigen to naive B cells. After recognizing the antigen, DCs migrate to secondary lymphoid organs, where they process and present it via MHC class I and II pathways. This interaction stimulates naive T cells to differentiate into CD4+, CD8+, and follicular helper T (Tfh) cells [31]. Tfh cells are essential for B cell maturation and antibody affinity, as they secrete interleukin-21 (IL-21), which promotes B cell proliferation and differentiation into plasma cells in GCs, ultimately leading to the production of high-affinity antibodies targeting the spike protein. SARS-CoV-2 mRNA vaccines predominantly induce a Th1 immune response, associated with antiviral activity, rather than a Th2 response, which is linked to allergic reactions. Studies of convalescent plasma from recovered COVID-19 patients reveal high levels of Th1 and Tfh cells, both critical in supporting the neutralizing antibody response [29].

Clinical studies have shown that the geometric mean titer (GMT) of neutralizing antibodies against the wild-type SARS-CoV-2 and variants such as Alpha, Delta, Beta, and Omicron were 546, 331, 172, 40, and 8, respectively. Corresponding inhibition rates were 100%, 100%, 93%, 90%, and 30%. However, booster doses significantly increased GMT levels, with wild-type and Omicron titers rising to 6,241 and 1,195, respectively. In convalescent individuals who received a single vaccine dose, neutralizing antibodies against wild-type and Omicron increased to 7,997 and 1,549, respectively. These findings highlight the critical role of booster doses in enhancing neutralizing antibody titers against both wild-type SARS-CoV-2 and the Omicron variant, though neutralization efficacy for Omicron remains approximately five times lower than for the wild-type virus [32,33].

Conclusion

The modifications to mRNA in vaccines have significantly enhanced the production of neutralizing antibodies by improving translation efficiency and enabling evasion of the innate immune response. Key optimizations, such as the substitution of uridine with N1-methyl-pseudouridine (1mψ) and the use of lipid nanoparticles (LNPs) as a delivery system, have been crucial to the success of mRNA vaccines. The BNT162b2 and mRNA-1273 vaccines, which demonstrated efficacy rates of 95% and 94.5%, respectively, target the receptor-binding domain (RBD) of the SARS-CoV-2 spike protein, primarily stimulating B cells to produce neutralizing antibodies. The adaptive immune response, critical for antibody production, is initiated by antigen presentation through major histocompatibility complex (MHC) class I and II molecules. CD8+ T cells recognize antigens via MHC class I and destroy infected cells, while CD4+ T cells recognize antigens via MHC class II and assist in B cell maturation. These B cells differentiate into plasma cells, which secrete neutralizing antibodies. As mRNA functions as an endogenous antigen, it effectively activates both CD4+ and CD8+ T cells, closely mimicking the natural infection process of SARS-CoV-2.

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Conceptualization, SZ and BED; Writing – Original Draft, SZ; Writing – Review & Editing, SE and BED.

Declaration of interest

The authors declare no conflict of interest.

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