mRNA Vaccinations for the Prevention of Viral Infections

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Abstract

mRNA vaccines for the prevention of infection disease have gained significant traction in the last three decades, specifically with the recent emergency approval of two COVID-19 vaccines (Oliver, Sara E et al., 2020; Rauch et al., 2018a). mRNA vaccines occupy a unique space in immunology because of their utilization of cellular mechanisms to produce viral proteins, and their simplicity and ease of manufacture (Cullis & Hope, 2017). These vaccines have shown strong humoral and cell-mediated adaptive immune responses (Rauch et al., 2018b) . In addition, side effects of the vaccines have been limited and subject response is manipulatable by varying the lipid nanoparticle (LNP) vector of the vaccine (Sedic et al., 2018; Zhang, N. et al., 2020). This thesis will provide an in-depth review of the types, mechanisms, and safety of mRNA vaccines, as well as a history of mRNA technology and the current advancements of the field.

mRNA Vaccinations for the Prevention of Viral Infections

Vaccines have saved countless lives over the centuries they have been in use, and vaccinology is expanding as new technology and techniques are applied. Common forms of widely used vaccines are live-attenuated viruses and inactivated viruses, which are weakened forms of the virus that help the immune system of the body to recognize the virus without the pathogenicity that actual infection would cause (*Vaccine Types,* 2017). Attenuation is the process of mutating or weakening the virus to a form that is similar to the virus so that the immune system creates antibodies against proteins found in the virus but does not rapidly replicate and harm the body (Morrison & Plotkin, 2016). Like attenuated vaccines, messenger RNA (mRNA) vaccines provoke an immune response by coding for an antigen that the target virus expresses but isolated from the rest of the virus will not present illness (Zhang, C. et al., 2019). mRNA viruses are commonly found in lipid coating to protect the mRNA from degradation in the body, although there are several methods of mRNA delivery in use (Cullis & Hope, 2017).

mRNA vaccines have shown great potential in stimulating immune cells and responses are highly manipulable through gene editing for specific protein epitomes. Recently, COVID-19 mRNA vaccines have entered circulation and have shown great success at preventing infection (Oliver et al., 2020; Oliver, Sara E., 2021). mRNA vaccinology continues to expand, proving in many cases to be more cost-effective and easier to produce than their counterparts (Jackson, N. A. C. et al., 2020). Recent studies in self-amplifying mRNA vaccines, which can use vaccine transcriptional proteins to increase antigen expression, have shown high levels of immunogenicity with fewer vaccine particles compared to conventional mRNA vaccines (Stokes et al., 2020). This thesis will examine the types, mechanisms, history, and safety of mRNA vaccines, as well as assess the current state of mRNA vaccinology.

History of mRNA Vaccination

Vaccination is a centuries old practice, originating with the work of Edward Jenner to vaccinate for smallpox and Louis Pasteur's Germ Theory (Zhang et al., 2019). These vaccines were simple but remarkable, using various methods to familiarize the body to a pathogen without overwhelming the immune systems defense. Jenner and Pasteur did not even know the structure or nature of the bacteria and viruses under investigation, although effective treatments were developed through observation and trial and error. Vaccination has helped the world through dark times and has helped with the eradication of several diseases (Hajj Hussein et al., 2015). However, some diseases continue to evade defeat via vaccination, thus there remains a continuous effort to develop new vaccination methods and find unique strategies to outsmart the new and emerging pathogens. These persistent pathogens such as HIV (human immunodeficient virus) have been the catalysts for mRNA vaccine technology.

mRNA vaccine technology has followed DNA vaccines as well as subunit and vaccines. DNA vaccines suffer from complications due the additional step of transcription required before a protein can be produced, as well as safety concerns to their integration in the nucleus (Zhang et al., 2019). Subunit vaccines lacked the potent immune response that mRNA vaccines have been found capable of (Zhang et al., 2019). Another appeal of the mRNA vaccine was its simplicity, which has the possibility of quick manufacture during epidemic situations like COVID-19 (Pardi, Hogan et al., 2018).

The LNP was already in widespread use in studies to transport siRNA for gene silencing, so finetuning the lipids for mRNA was an easy task (Pardi et al., 2018). Groundbreaking research in the 1990s provided the understanding for mRNA modification that allowed mRNAs to be stable enough to facilitate effective protein expression (Zhang et al., 2019). Since then, research into

mRNA vaccines has increased exponentially. mRNA vaccination has also been spurred on by possibilities of cancer therapy, which represents the other main interest in mRNA vaccines aside from infectious disease (Fiedler et al., 2016). mRNA vaccines can produce any protein in the body if the sequence is known, which allows mutant cancer proteins to be targeted for the specific destruction of cancer line cells.

Prior to the vaccines to 2020's COVID-19 vaccines, no mRNA vaccine had been approved by the US Food and Drug Administration (FDA) (Oliver et al., 2020). However, due to the urgent need for a vaccine and funding under Operation Warpspeed, Pfizer/BioNTech's vaccine was approved for use on December 11th, 2020 (Sahin et al., 2020). mRNA vaccine technology continues to improve and now that the COVID-19 vaccines have set precedence for mRNA vaccine development, more mRNA vaccines are sure to follow.

Divisions of mRNA Vaccines

Current mRNA vaccines fall into the four following divisions or categories: naked mRNA vaccines, lipid nanoparticle (LNP) protected mRNA vaccines, charge-altering releasable transport (CART) mRNA vaccines, and capsid-coat mRNA vaccines. Each class has shown promise and utility in some capacity for preventing infectious disease, and selection of a vaccine type can be pathogen dependent.

Naked mRNA vaccines consist of a vaccine where the mRNA is not accompanied by any type of protective coat. These types of vaccines have been shown to be effective at producing protein expression in local tissues of mouse and human skin (Probst et al., 2007). In this manner, the protein is expressed on MHC (major histocompatibility complex) I molecules and targeted by helper and cytotoxic T cells. One downside of the naked mRNA vaccine is the lack of humoral response triggered. Unless the host cell secretes the translated protein of interest, there will not be

any B cell interaction and no antibody response (Sahin et al., 2014). Without the ability to quickly produce IgG antibodies through activated B cells, a severe infection could still occur after immunization.

There is evidence that there are specific mechanisms that control the transport of nucleic acids through the cell membrane in a transport process that is mediated by calcium, as an increased calcium concentration in the mouse vaccine by Probst et al. showed an increase in protein expression (Probst et al., 2007). This type of local injection of naked mRNA is somewhat limited in its effect because mRNA has a short half-life in the extracellular matrix and injection to a tissue group only produces local protein expression (Probst et al., 2007; Sorrentino, 1998). To stimulate an immune response, it is necessary for the translated proteins to be presented on MHC molecules so that T cells can be activated to produce adaptive immunity to the antigen, and therefore the associated pathogen.

Because the dermis lacks a high concentration of antigen-presenting cells (APCs), it is necessary to inject the mRNA into a tissue with a higher APC presence or utilize an adjuvant to effectively recruit greater amounts of dendritic cells and lymphocytes. For this reason, naked mRNA has also been used for stimulation of the mouse immune system intranodally (Kreiter et al., 2010). These intranodal injections of mRNA produce increased levels of T cells, interlukin 2 (IL2), and activated natural killer (NK) cells, as well as the mRNA-coded protein of interest (Kreiter et al., 2010). Additionally, the intranodal injections in mice were shown to have produced memory T cells against the antigen in question, conferring cell mediated immunity (Kreiter et al., 2010). The use of naked mRNA is limited, but intranodal injections show great potential for producing cell mediated immunity against specific antigens.

Because mRNA degrades rapidly in certain unfavorable conditions and is not suited for survival outside of its intended environment in the cell nucleus and cytoplasm, protecting the mRNA via a delivery vector can increase the immune response to the vaccine by ensuring more mRNA is taken up by the host cells. Therefore, just as a virus uses a protein or a lipid coat to protect its genetic material from the extracellular matrix, mRNA vaccines can be modified to better withstand the environment in the body (Sedic et al., 2018). A lipid nanoparticle coat has been used to protect mRNA particles for exogenous protein production in rat and monkey models with successful and safe treatment (Sedic et al., 2018). Historically, liposomes have been used to transport anti-cancer drugs to tumor tissues. These liposomes are particularly effective types of LNPs because of their size of <100 nm and the neutrality of the charge of the extracellular portion of the molecule, preventing the integration of proteins via charge-driven interactions (Cullis & Hope, 2017). As of 2017, there were 9 intravenous LNP drugs approved for treatment. Unfortunately, liposomes are ineffective at encapsulating genetic material due to the size and negative charge of the phosphate backbone of nucleic acids (Cullis & Hope, 2017).

Cationic lipids were proposed due to the positive charge they possess, which could associate with the negatively charged RNA (Cullis & Hope, 2017). Cationic lipids alone are unsuitable for in vivo genetic material delivery because they have a positive surface charge that causes instability and toxicity in the host (Cullis & Hope, 2017). Eventually, an ethanol-loading process was devised together with a mixture of lipids which gave the best of both worlds: an LNP with a cationic interior and a neutral exterior (Cullis & Hope, 2017). Polyethylene glycol (PEG) lipids are used to obtain the outer shell, while a mixture of structural lipids and cationic lipids form the inner, inverted capsules that surround the genetic material (Cullis & Hope, 2017). These LNPs were especially effective at delivering short interfering RNA (siRNA) to the liver because

hepatocytes express apolipoprotein E (ApoE), which has been shown to adsorb to the LNP and target it for endocytosis (Cullis & Hope, 2017). Once inside the cell, the cationic lipids are protonated and attract the anionic lipids of the endosome, causing the release of the genetic material into the cytoplasm (Figure 1). The effectiveness of this pathway into the cell is limited without ApoE, causing a significant roadblock for LNP-vector treatments in the future (Cullis & Hope, 2017).

Figure 1. LNP-facilitated mRNA release. The negatively charged mRNA associates with the cationic lipids during LNP formation. Upon phagocytosis, the low pH of the vesicle induces negative charges of the membrane lipids, causing a reaction between the cationic lipids of the LNP and the anionic membrane proteins, causing a fusing of the lipids and the release of the mRNA into the cytoplasm. (Cullis & Hope, 2017).

An alternative to LNPs are charge-altering releasable transporters (CARTs). CARTs are like LNPs in that a charge-directed mechanism is used to release their contents into the cytoplasm. Oligo (carbonate-*b*-α-amino ester)s are the molecules that make up the complex with negatively

charged mRNA (McKinlay et al., 2018). The CARTs are complexes where the cationic amino groups stabilize the large, negative mRNA molecules, allowing the mRNA to travel through the body in a protected fashion (McKinlay et al., 2017). Once the CART complexes have reached the membrane, endocytosis occurs, and under a pH dependent reaction the amino groups are neutralized to amide groups, losing their positive charge and ability to stabilize the mRNA, causing the dissociation of the mRNA molecules (McKinlay et al., 2017). The amide groups detach and cyclize to form small neutral molecules that are easy and safe for the host cell to degrade, resulting in an efficient mRNA delivery without some of the toxic side effects of LNP delivery (McKinlay et al., 2017).

The manipulation of the type of lipid and the number of carbonate and amino groups used can be manipulated to suit the target cells or the size of mRNA being transfected (Haabeth et al., 2018). Additionally, the ratio of CART molecules to mRNA molecules can be manipulated for the best rates of translation, based upon the treatment type and the desired effect (McKinlay et al., 2017; McKinlay et al., 2018). Another useful aspect of the CART delivery method is the ability to incorporate adjuvant directly into the delivery complex, to ensure the highest doses of adjuvant to the cells receiving the mRNA for protein translation. Addition of CpGs, promotor regions with cytosines followed by guanines, increases immunogenicity of the vaccine. CPGs are recognized by Toll-like receptor-9 (TLR9), an receptor that adheres to common viral RNA sequences, which increases the APC expression of the translated antigen by MHC molecules and causes a strong cytotoxic T cell response (Haabeth et al., 2018).

A capsid coat is a protective layer of proteins that some viruses use to protect their genomic material. The viruses can self-replicate by encoding the proteins that constitute the protein coat and replicate in infected cells. This virus machinery can also be used to create mRNA vaccines that are

able to infect cells efficiently, but the mRNA can be manipulated so that no further infectious particles form, because the propagation of these virus particles is responsible for the fever, inflammation, and other symptoms associated with infection. One study was conducted with the Semliki Forest Virus (SFV) vector, to confer immunity to an influenza strain in mice (Zhou et al., 1994). The authors of the study infected cell cultures with two mRNA strands simultaneously, one coding SFV proteins and the other coding influenza protein (Zhou et al., 1994). The mRNAs were manipulated so that only the influenza protein was tagged for incorporation into the capsid coat (Zhou et al., 1994). This modification insured that these new virus particles would not continue to replicate in newly infected cells (Zhou et al., 1994). In mice, these new virus particles were successful in producing anti-influenza IgG antibodies and cytotoxic T-lymphocytes (Zhou et al., 1994). Proteins that make up the capsid coat of a virus can be effective targets for the immune system since these proteins are exposed and accessible to cells of the immune system.

Mechanisms

To effectively serve as vaccine agents, mRNA vaccines need to facilitate effective delivery to the cytoplasm of target cells, whether they be APCs such as dendritic cells or muscle tissue. As most vaccines are delivered intramuscularly, the muscle tissue and surrounding immune cells are the targets of the vaccine. LNP-encapsulated vaccines function by undergoing endocytosis by the target cells. Once encapsulated by the target cell, the cationic lipids which form the LNP interact with the negatively charged endosomal membrane to facilitate the release of the contents of the LNP (Tan & Sun, 2018). The low pH of the endosome causes the ionization of the cationic lipids; without the low pH of the endosome to facilitate release of the mRNA, the LNP would not be triggered to release the mRNA (Tan & Sun, 2018). If the LNP were ionized at too high a pH, it

would be too unstable to be dependable and could deteriorate at physiological pH, failing to reach the target cell cytoplasm (Tan & Sun, 2018).

The idea behind the ribosomal translation of the vaccine-borne mRNA is that by the time the mRNA has been released from the LNP, it is indistinguishable from the host's own mRNA (Wadhwa et al., 2020). Thus, the cell will indiscriminately translate the mRNA into its coded peptide chain and fold the protein as if it were a native protein. Although the mRNA can have modified bases, tails or caps, because these structures are modeled after human mRNA, the cell machinery is able to effectively translate and fold the *in-vitro* synthesized (IVT) mRNA (Wadhwa et al., 2020). To boost the half-life of the mRNA during this naked stage, the mRNA can be modified using tails or caps to avoid rapid degradation by RNase. These modifications will be explored in more depth later.

Once the vaccine antigen has been translated by the cell, the cell will attempt degrade the protein via proteosomes, because the protein will not contain a signal peptide (Wadhwa et al., 2020). The signal peptide is the cells normal mechanism of determining the destination of the newly synthesized protein: cell membrane, extracellular matrix, or cytoplasm. The proteosome will degrade the protein into its peptide epitomes, which will be bound by MHC class I molecules, which, in turn, present the antigen at the surface of the cell and allow for the recognition of the antigen by T cells, with their matching T-cell receptors (TCRs) (Wadhwa et al., 2020).

To have an effective antibody response via humoral immunity, it is necessary for the antigen to exit the cell and be taken up APCs with MHC II. Once the antigen epitomes are bound to MHC II, recognition by naïve B cells is possible and a humoral response can be mounted against the antigen (Rauch et al., 2018). One mechanism for this MHC II expression is through the phagocytosis of the infected cell via an APC, such as a dendritic cell (Rauch et al., 2018). Because

the mRNA and other vaccine components trigger the TLRs of the cell, other immune cells such as dendritic cells are recruited to deal with the potential infection and to phagocytose compromised cells.

Once the cell has been phagocytosized, the contained antigen can be processed and presented on MHC class II molecules to produce the humoral immunity response, which will enable antibody production against the pathogen (Rauch et al., 2018). In addition to the phagocytic pathway of dendritic cells, a secretion peptide signal can be attached to the mRNA sequence of the antigen (Sahin et al., 2014). Upon folding the protein, the cell will package the antigen for release to the extracellular matrix, where in can be encountered by APCs capable of MHC II expression (Sahin et al., 2014).

Adjuvants are elements that are added to a vaccine to enhance the immune response to the vaccine. By amplifying the response, a more complete protection can be afforded, possibly negating the need for additional rounds of vaccination or booster shots. Adjuvants function by leveraging the mechanisms the innate immune response has available to detect and counter pathogens. To detect the presence of foreign invaders, the body uses pattern recognition receptors (PRRs) to recognize common invasive agents. PRRs such as Toll-like receptors (TLRs) can identify proteins or other molecules that are unique to bacteria or viruses that are not expressed by the body, to avoid auto-immune responses (Edwards et al., 2017). Genetic material can also serve as an activator of TLRs, so genetic material in the form of an mRNA vaccine can serve as a self-adjuvant to TLRs 7 and 8 (Kawai & Akira, 2009). This feature explains the efficiency and simplicity of mRNA vaccines; their very own genetic material increases their immunogenicity, mimicking virus pathology. One study performed with hemagglutinin mRNA from influenza A showed that TLR activity was upregulated in association with mRNA vaccine injection in mice,

supporting the theory that TLRs are involved in the immunogenicity of mRNA vaccines (Pardi, Parkhouse et al., 2018).

Additionally, it is possible to add additional small molecules to the vaccine which produce more potent immune responses. One study, utilizing the TLR activator R848 incorporated into a mRNA vaccine, found heightened specific cell-mediated immune response to the mRNA in question (Islam et al., 2021). Cytosine phosphoguanine (CpG) synthetic bases have been thoroughly utilized as vaccine adjuvants, and are recognized by TLR9, which is an endosomal PRR that is prevalent in dendritic cells which are crucial in adapted immunity through MHC presentation (Coffman et al., 2010).

Another form of mRNA protection is nucleotide substitution. Pyrimidine nucleotides such as 1-methylpseudouridine triphosphate and 5-methylcytidine triphosphate have been used as substitutions for the uracil and cytosine respectively to prevent recognition by TLRs (Sedic et al., 2018). RNase is secreted by the pancreas and various other tissues throughout the body to degrade RNA specifically in the body, by catalyzing the hydrolysis of the phosphodiester bonds that form the backbone of the RNA molecule (Sorrentino, 1998). These RNase enzymes contribute to the short half-life that mRNA molecules display in the cytoplasm. The mechanisms to protect mRNAs and inhibit the activity of RNase enzymes in the cytoplasm of the cell are numerous and complex, and these mechanisms are dependent on the post-transcriptional modification of the mRNA (Houseley & Tollervey, 2009).

The addition of a Poly-A tail, a 150-250 nucleotide repetition of adenine nucleotides, at the 3’ end of the molecule is one mechanism that the body uses to increase the stability of the mRNA (Houseley & Tollervey, 2009). Polyadenylation has been used during modification of exogenous mRNA to increase its half-life in the cytoplasm of the cell. Untranslated region (UTR)

modification on the 3’ and 5’ ends of the coding regions can be used to increase translation rate and regulate the transport of the newly formed protein (Sahin et al., 2014) Eukaryotic mRNA can be post-transcriptionally modified to contain a 7-methylguanosine cap which aids in translation by its interaction with translation initiation factor 4E (Sahin et al., 2014). Using the viral enzyme mRNA guanyl transferase, addition of a 7-methylguanosine cap to the 5’ end of IVT mRNA can be achieved, mimicking the structure of native post-transcribed mRNA without incorporation of the IVT mRNA into the cell’s nucleus (Martin et al., 1975).

Coronavirus

The U.S. government worked to fund 5 vaccine candidates to prevent the spread of COVID-19 under an initiative termed Operation Warp Speed ((ASPA), Assistant Secretary for Public Affairs, 2020; Brüssow, 2020). Out of the five vaccine candidates which were part of the program, two were classified as mRNA vaccines (Brüssow, 2020). Moderna and Pfizer developed these two vaccines that utilized mRNA coding for the same spike protein (S), to stimulate an immune response (Brüssow, 2020). The methods used by these developers to effectively stimulate an immune response is discussed below.

Both BioNTech/Pfizer and Moderna vaccines use the same general formula: a LNP-encapsulated mRNA strand encoding the 1,273 amino acid spike protein. Moderna’s vaccine, mRNA-1273, consists of mRNA for the S-2P antigen from SARS-CoV-2 (the novel COVID-19) virus (Jackson, L. A. et al., 2020). The basic structure of the vaccine is an LNP-encapsulated mRNA diluted with saline (Jackson et al., 2020). A mixture of 4 lipids was used, although the formula of the lipids was not disclosed in the preliminary Phase 1 trial report (Jackson et al., 2020).

Phase 1 trials were initiated in a record time frame, due to the current crisis caused by COVID-19 (Chung et al., 2020). The vaccine manufacturing-process used several techniques

previously mentioned, and mRNA substitutions were made so that a particular stable conformation of the S protein would be expressed which would stimulate a strong immune response to the native protein found on the SARS-CoV-2 virus (Graham et al., 2019; Jackson et al., 2020). The S protein has a sequence of 1,273 amino acids, with a 222 amino acid receptor-binding domain (RBD) which associates with angiotensin-converting enzyme 2 (ACE2) in humans (Hu et al., 2020). Other known non-pathgenic SARS-CoV viruses differ in some residues in the receptor binding domain (RBD); these mutations in SARS-CoV-2 increase the affinity of S protein for the ACE2 protein (Hu et al., 2020). The mRNA-1273 vaccine contained a modified mRNA code where the amino acid residues at positions 986 and 987 had been changed to proline residues to cement the translated protein in the prefusion conformation, as the protein undergoes a conformational change when bound to its receptor (Chung et al., 2020; Graham et al., 2019; Jackson et al., 2020). mRNA-1273 has a modification in one of the RBD domains of the S protein to maintain the prefusion conformation (Figure 2).

Isolating the protein in its prefusion state is imperative because the antigen encoded by the mRNA must have the same structure as the conformation of the virus protein upon viral entry so that antibodies created can neutralize the virus. Moderna does not report the use of an adjuvant in their vaccine, although mRNA itself functions as a PAMP through recognition by TLRs 7 and 8, perhaps increasing the immunogenicity of the vaccine. Additionally, some LNPs can be potent adjuvants, so it is possible that Moderna utilized lipids as an adjuvant for their vaccine (Perrie et al., 1987). Moderna also mentions chemical modification of the mRNA to prevent premature detection by the body, although the nature of the modifications is not specified (Jackson et al., 2020). Perhaps some nucleotide substitutions were used as previously described to prevent elimination by TLRs (Sedic et al., 2018).

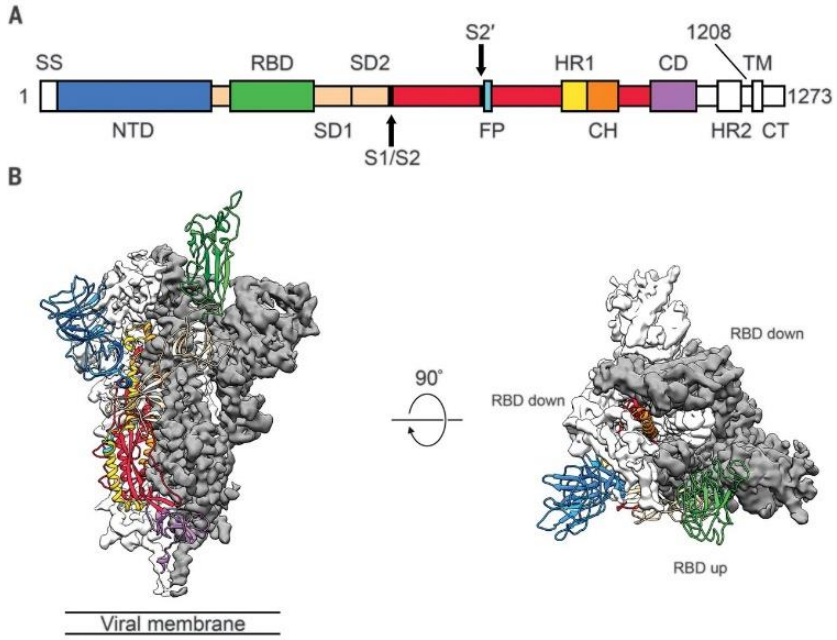

Figure 2. COVID-19 S protein structure. The structure of the spike protein of the COVID-19 virus was obtained via cryo-electron microscopy (cryo-EM) imaging, and the results were published on the 13th of March 2020, allowing vaccine manufacturers to quickly tackle the task of vaccine production (Wrapp et al., 2020). The spike protein is a trimeric protein, with its subunits denoted as S1, S2, and S3 (Wrapp et al., 2020). The conformation as seen above is in the prefusion state, which is distinguishable from the other conformations of the protein by the position of the RBDs. The S1 RBD (green) undergoes a hinging motion upon receptor binding that facilitates viral adhesion (Wrapp et al., 2020). "Structure of 2019-nCoV S in the prefusion conformation" by Wrapp et al. is licensed under CC BY 4.0.

The U.S. Food and Drug Administration (FDA) approved use of the Moderna vaccine on December 18th, 2020 (Oliver, 2021). This was after reviewing the phase III vaccine trial that was carried out in a double-blind study of 30,000 participants, across race, age, and gender, and secondary medical conditions were also present (Mahase, 2020). The trial resulted in a 94.1% efficacy rate at preventing symptomatic infection of COVID-19 (Oliver, 2021). The vaccine is also

likely to offer protection from asymptomatic transmission, which is important for the development of herd immunity to the virus. Due to the recent nature of the study, it is impossible to tell the length of immunity and whether booster vaccines will be required for continued protection to the vaccine. The symptoms of the vaccine itself were mild, with only 1% of participants reporting severe reaction (Oliver, 2021). The vaccine requires two doses, spaced 4 weeks apart (Oliver, 2021). One benefit of the Moderna vaccine is that it can be safely stored in a freezer for long-term storage or in a refrigerator for up to 30 days, which aids in the distribution of the vaccine (Mahase, 2020).

The BioNTech-Pfizer vaccine, BNT162b2, is also an LNP-encapsulated mRNA vaccine encoding the spike protein in its prefusion state (Walsh et al., 2020). Pfizer's vaccines contain the lipids ALC-0315 and ALC-0159 and cholesterol (Polack et al., 2020). ALC-0135 serves as the cationic lipid, assisting in the formation of the LNP around the negatively charged mRNA (Polack et al., 2020). Inside the cell, ALC-0315 allows the mRNA to be released from the endosome due to electrostatic interactions between the lipid and the endosomal membrane (Polack et al., 2020). ALC-0159 is the structural PEG lipid for the LNP, and cholesterol also aids in the formation and regulation of the size of the LNP (Polack et al., 2020). BioNTech-Pfizer were originally researching 4 different vaccines for COVID-19, including BNT162b1, which contained only partial S protein code (Walsh et al., 2020). Although BNT162b1 resulted in similar immune response compared to BNT162b2, BNT162b2 was pursued in phase II/III trials due to its lower symptomatic response in participants during phase I trials (Walsh et al., 2020). BNT162b2 also contained N1-methylpseudouridine nucleosides to increase the half-life of the mRNA in the cell cytoplasm (Polack et al., 2020). Pseudouridine, in place of uridine, offers higher translational capacity and avoids or limits detection by TLRs (Karikó et al., 2008). Research shows that

BNT162b2 also confers immunity to 19 different pseudoviruses, with unique mutations to the RBD of the virus (Sahin et al., 2020). If COVID-19 can perform antigenic drift, leading to mutations of the S protein, it is imperative that the vaccine offers protection to novel strains of S protein, so this data is very encouraging.

The BioNTech-Pfizer vaccine was approved for use by the FDA on December 11th, 2020, less than 11 months after the genetic information of the coronavirus was released (Oliver et al., 2020; Polack et al., 2020). Like Moderna's vaccine, BNT162b2 requires two doses, with 28 days between doses (Oliver et al., 2020). This vaccine has a reported efficacy rate of 95% in its phase III trial, with 43,548 total participants, with diverse race, gender, ethnicity, and health conditions represented (Oliver et al., 2020). The efficacy rate among all the distinguished subgroups was above 92%, and serious adverse effects were below 1% (Polack et al., 2020). The BioNTech-Pfizer vaccine requires storage at −70°C, making it more difficult to transport and distribute compared to mRNA-1273 (Mahase, 2020).

Safety and Ethics

The novelty of mRNA vaccination combined with the emergency approval of the recent COVID-19 mRNA vaccines has left questions as to whether these vaccines are safe and if there are detrimental side-effects to the vaccines. mRNA vaccines are introducing the body to foreign material including modified nucleosides and lipids (Jackson et al., 2020). The immune system does recognize the material as foreign which is the reason for the inflammation or pain that is sometimes associated with vaccination (Sedic et al., 2018). These are necessary for immune system stimulation, and if the lipids and mRNA modifications are present in the correct concentrations, the danger of adverse reactions can be mitigated. The safety risks associated with mRNA vaccines are

inherent in all vaccine forms; vaccines necessarily must introduce foreign material into the body to stimulate an immune response.

Although mRNA vaccines are introducing the body to an element of a pathogen, there is no danger of contracting the disease since the mRNA is only coding for a single protein or a subunit of a single protein. There is no risk of viral infection associated with mRNA vaccines. Another benefit of mRNA vaccines is the body's eventual elimination of the mRNA. Because viruses often have single or double-stranded RNA genomes, the body has RNAase and TLRs to recognize and neutralize foreign mRNA (Sahin et al., 2014). This makes mRNA inherently fragile and short-lived in the cytoplasm of cells. mRNA modification such as poly-A tails and nucleoside modification give vaccine developers some room for modification to fine-tune the half-life of mRNA in vaccines (Jackson et al., 2020). If the vaccine is well-tolerated, the longer the half-life the better because more protein will be produced, allowing for greater immunogenicity.

Because the cells which are infiltrated with mRNA will present the material on MHC I or II molecules, these cells with be targeted for apoptosis by the immune system (Zhang et al., 2019). Unlike viruses, however, there are a limited number of mRNA molecules injected, and these molecules cannot propagate like viruses. To create an effective, safe vaccine, the balance between safety and strong immune system simulation must be struck. By modifying the lipid structure and LNP size, safe and effective vaccines can be produced, as demonstrated by recent COVD-19 mRNA vaccines.

A phenomenon known as antibody dependent enhancement (ADE) complicates the functionality of some vaccines (Arvin et al., 2020). In some viruses, the binding of antibody to the virus at the Fab region (the antigen-binding fragment of immunoglobins) can enhance the affinity of cell receptors for viral antigens (Arvin et al., 2020). In cases like these, antibody formation to a

viral antigen can enhance the pathogenicity of a virus during a secondary infection (Arvin et al., 2020; Lazo et al., 2007). To protect against these pathogens, targeting cell-mediated immunity is preferred, since cell-mediated immunity offers specificity without the use of antibodies. One study explored the ability of infection of one of the protein coat serotypes of the dengue virus to protect against the dengue virus (Lazo et al., 2007). This protein-based vaccine was preferred for the dengue virus since the protein coat of the virus is surrounded by a lipid membrane embedded with glycoproteins (Lazo et al., 2007). Since the capsid protein is not exposed on the surface of the cell, no humoral response to the protein occurs; only MHC presentation, leading to a cell-mediated response (Lazo et al., 2007). The potential for an ADE response to a vaccine complicates the formation of some mRNA vaccines. The mechanisms of ADE are not well understood, and further studies need to be done to understand the inconsistencies that have been seen in the study of ADE for certain pathogens.

Current Developments and Future Opportunities in mRNA Vaccine Technology

The recent success of COVID-19 mRNA vaccines has shown the scientific community that mRNA vaccines are not only viable drugs, but that under the right conditions, mRNA therapeutics can be synthesized quickly and economically due to their relative simplicity. The COVID-19 vaccines discussed previously have opted for LNP vectors of mRNA vaccine delivery, which seems to be ideal for intramuscular delivery, which has been conventional for almost all injectable vaccines. In the future, however, there may be the possibility of intravenous or intranodal injections, which could be used to target specific tissues or systemic vaccine delivery (Huang et al., 2020). Because most research up to this point regarding mRNA vaccines has used intramuscular vaccination, these other administration routes could be researched. Perhaps LNPs will remain

effective vectors when tested in new tissues, although it is possible that LNPs will need adjusting or new delivery routes will need to be explored (Huang et al., 2020).

Among the exciting new advancements of mRNA vaccines is the potential for making new vaccines to the seasonal flu. The current flu vaccines are inactivated or live-attenuated and are more difficult and time-consuming to produce than mRNA vaccines (Alameh et al., 2020). Additionally, a recent study showed that an mRNA vaccine in mice, targeting the hemagglutinin spike of the influenza virus, was able to offer protection against more than one unique strain of the flu (Pardi et al., 2018). This broader protection of the vaccine would be valuable given the volatility of the flu proteins (Alameh et al., 2020). Due to the annual nature of the flu season, the rapid timeline of mRNA vaccines would be invaluable. Once an effective formula for the flu is prepared, fighting the flu could become a case of "plug-and-chug" vaccine manufacturing that would only vary in the specific mRNA sequence for the current year.

mRNA vaccine technology is also being tested for use against HIV, although it has proven difficult to produce neutralizing antibodies to HIV envelope protein through any vaccine model (Alameh et al., 2020). One recent study was able to produce high levels of antigen-specific IgG in monkeys through mRNA vaccination, but despite the high titers against the envelope protein, the effective immune response was limited to half of the vaccine subjects (Pardi et al., 2019). Future research is certainly needed regarding HIV, due to the evasive nature of the virus.

Among the other recent advancements in mRNA vaccine development is a potential vaccine against the herpes simplex virus type 2 (HSV-2), the STD behind genital herpes. A 2019 study in guinea pigs using a trivalent mRNA vaccine which encoded three separate virus glycoproteins showed that high titers of antibodies against each of the proteins was achieved (Awasthi et al., 2019). This vaccine had higher titers than other subunit vaccines that proceeded to

human trials, so this vaccine has the potential to become the first vaccine against HSV-2 (Awasthi et al., 2019). The multivalent character of this vaccine is also worth noting because other vaccines with multiple different surface proteins could also be attacked in a similar multivalent fashion, increasing the protection afforded by the vaccine. The flu vaccine particularly could be targeted in this fashion since the hemagglutinin and neuraminidase proteins each contribute to the virus's pathogenicity and are specific antibody targets.

Self-amplifying viruses were mentioned previously, although the self-replication mechanism is promising and worth examining in greater detail. The basic premise of the self-amplification process is using virus structures and mechanisms to promote vaccine efficacy (Maruggi et al., 2019). RNA viruses in their wild-type form contain cellular machinery to often interfere with cell function or to promote the translation of virus mRNA over cell mRNA (Maruggi et al., 2019). Self-amplifying mRNA vaccines contain mRNA for structural virus proteins that facilitate the promotion and replication of virus mRNA (Maruggi et al., 2019). In this manner, self-amplifying viruses can produce several times the number of mRNA of interest compared to conventional mRNA vaccines, increasing the immune response to the protein. Although self-amplifying vaccines are using virus technology to amplify, the virus proteins that cause the propagation of additional virus particles that are associated with viral infections have been removed or mutated so that there is no risk of infection (Stokes et al., 2020). The key differences between conventional and self-amplifying mRNA vaccines can be seen in Figure 3.

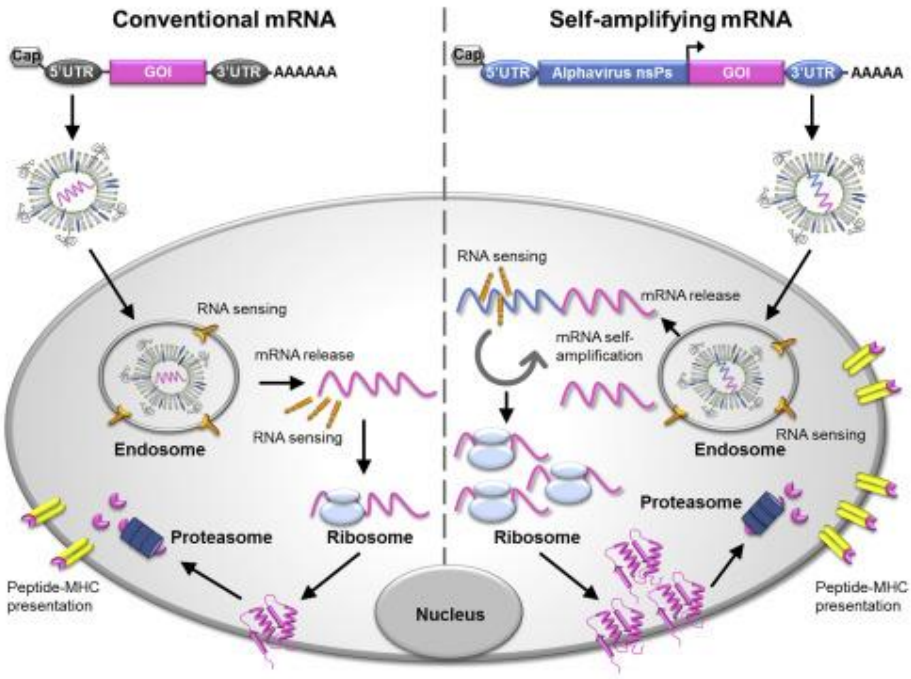

Figure 3. Conventional mRNA vaccine vs Self-Amplifying mRNA. Self-amplifying mRNA vaccine particles share similar structures with convention mRNA particles, with the key difference being the presence of additional virus-derived mRNA coding for replicating proteins, indicated above as nsPs (non-structural proteins). Once the cell's machinery has translated these replicating proteins, the proteins for replicating centers that are able to duplicate the mRNA of interest so that cellular ribosomes can translate all these copies into the protein of interest (Maruggi et al., 2019). "Schematic Representation of mRNA Vaccines and Mechanism of Antigen Expression" by Maruggi et al. is licenced under CC BY-NC-ND 4.0.

One 2020 study on a self-amplifying mRNA vaccine for rabies in rats showed that the vaccine had no significant increased risk to the rats compared to responses to conventional vaccines (Stokes et al., 2020). In addition, the protein-of-interest mRNA was found in the lymph nodes of the rats 1-day post-immunization, indicating that the vaccine had been transported to the lymph nodes, which is where APCs present antigen to B and T-cells (Stokes et al., 2020). An IgG response to the protein-of-interest was detected in the rats, indicating that the vaccine had

stimulated the expected immune response (Stokes et al., 2020). More research needs to be done with self-amplifying mRNA vaccines, but the technology is showing promise, and if a formula is perfected these vaccines could possibly require much smaller doses than conventional mRNA vaccines while maintaining high immunogenicity. This experiment was carried out in rats so there is still significant progress to be made before this vaccine structure can be used for protection against viral infections in humans.

Recently there have also been studies done concerning the generation of monoclonal antibodies from mRNA delivered as a passive mRNA vaccine. DNA-mediated antibody treatments have been tested in the past but suffer from greater safety issues than mRNA vaccines (Thran et al., 2017). One study looked at the efficacy of anti-rabies and anti-botulinum antibodies in a prophylactic and therapeutic administration (Thran et al., 2017). The study showed that the encoded antibodies were effective at preventing the death of infected mice in both prophylactic and therapeutic delivery schemes (Thran et al., 2017). In the case of botulinum toxin, after an administration of toxin $4xLD_{50}$, 100% of the mice survived when the vaccine was administered 6 hours after the initial infection (Thran et al., 2017). The study also detected antibodies in as little as 2 hours post-treatment (Thran et al., 2017). Future research into mRNA mediated antibody treatment is still needed, although preliminary research shows promising results. Combining mRNA-mediated antibody treatment with self-amplifying mRNA technology could lead to high serum antibody levels that could be effective at preventing or therapeutically treating viral infections.

Conclusion

mRNA vaccines occupy a unique space in vaccinology: these vaccines are simple, cheap, and are easily produced for nearly any antigen of interest. The modifiable LNP shells and potent

immunogenicity make these vaccines potentially viable vaccine candidates for several viral agents. With the emergency approval of the two COVID-19 vaccines, mRNA vaccines are finally being used for disease prevention, with great preliminary success. Self-amplifying mRNA vaccines are still in the early stages of research, although they show promising immunogenicity data. In the future, mRNA vaccines toward the flu may offer broader protection and faster synthesis than current vaccine options. Although still early in development, mRNA vaccines are gaining in popularity and are experiencing broad application due to COVID-19.

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