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LIPID-BASED NANOPARTICLES FOR DELIVERY OF VACCINE ADJUVANTS AND ANTIGENS: TOWARD MULTICOMPONENT VACCINES

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ABSTRACT

Even though vaccine adjuvants have advanced significantly, there are still unmet demands that might allow for the creation of vaccinations that are appropriate for more difficult-to-treat infections (such HIV and TB) as well as cancer vaccines. Because of their adaptability, liposomes have previously demonstrated great efficacy as adjuvant/delivery systems and are probably going to find more applications in this field. The recent licensing of COVID-19 vaccines that contain lipid nanoparticles with encapsulated mRNA highlights the wide range of possibilities of lipid-based delivery methods. This article offers a summary of the many methods that may be assessed for the development of lipid-based vaccination adjuvants and delivery systems for antigens based on proteins, carbohydrates, and nucleic acids, along with the information on how these methods could be coupled to create multicomponent vaccines. It also highlights on the ongoing evolution of vaccine technology and the critical role lipid-based systems are expected to play in advancing vaccine development for both infectious diseases and cancer.

KEYWORDS: Immunization, Thermostable, COVID- 19, Immunogenicity, Liposomes, Encapsulation, Conjugation.

INTRODUCTION

In recent years, alternative approaches to vaccine development have emerged, focusing on pathogen subunits. These subunit vaccines, which can include proteins, carbohydrates, or peptides, have shown significant progress. Despite their enhanced tolerance and safety compared to traditional vaccines, subunit vaccines are frequently less immunogenic because they lack the pathogenic features of the original organism.[1]

Nucleic acid-based (DNA and RNA) and vector-based (e.g., adenovirus) vaccines offer a different approach by mimicking a live infection. These vaccines cause the expression of antigens in situ after immunization, thereby priming both B and T cell responses.[2] Recently, two mRNA-based vaccines developed by BioNTech/Pfizer and Moderna/National Institute of Allergy and Infectious Diseases (NIAID) became the first mRNA vaccines to receive conditional approval for human use. Following these, adenovirus-based vaccines from AstraZeneca, Johnson & Johnson, and Moscow-based Gamaleya were also approved to address the challenges posed by the COVID-19 pandemic.[3] [4] There are still a number of unmet medical requirements for better vaccines, particularly against challenging bacterial and viral pathogens. Examples include:

- ***Bacterial Pathogens***: Mycobacterium tuberculosis
- ***Viral Pathogens***: Respiratory Syncytial Virus (RSV), Cytomegalovirus (CMV), Zika virus, and Human Immunodeficiency Virus (HIV).[5]

For these pathogens, so far, there have been no vaccines that are efficacious, or only vaccines that are of limited efficacy. Utilizing the right delivery methods and adjuvants is essential to overcoming these obstacles as well as gaining a better knowledge of pathogenicity or tumor initiation and progression. Antigen delivery in particular can shield the antigens from deterioration, make it easier for antigen-presenting cells (APCs) to absorb them, and encourage their complete activation in order to start strong anti-vaccine Th1/cytotoxic T lymphocyte (CTL) responses and long-term immunological memory.[6]

By improving antigen presentation and/or stimulating the innate immune system through the detection and activation of certain cell receptors, adjuvants often increase immunogenicity and may provide long-term protection against infections. When developing vaccines, insoluble aluminum salts have been the most often utilized adjuvant. Nevertheless, drawbacks have included some antigens' incompatibility or inefficiency as well as their inability to trigger potent cell-mediated immune responses, particularly cytotoxic T-cell reactions, which has led to the hunt for substitute adjuvants. Although many adjuvants function as both delivery mechanisms and immunopotentiators, adjuvants have been generally categorized into these two categories.[7]

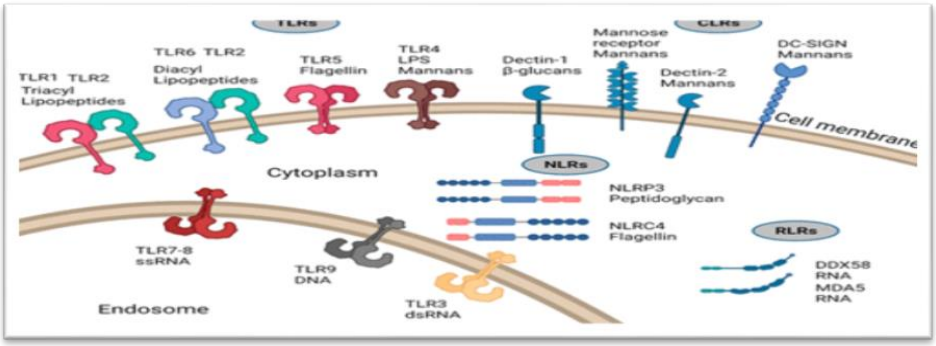


Figure 1: Schematic representation of the major PRRs and their corresponding ligands.

Next generation of vaccines will likely benefit from combination adjuvant approaches, which target multiple branches of the immunological reaction simultaneously. Adjuvants can act through various mechanisms such as creating a depot effect, inducing cytokines and chemokines, recruiting immune cells, enhancing facilitating antigen transport to draining lymph nodes and enhancing antigen absorption and presentation.[8] Combining different adjuvants can harness these diverse immunostimulatory properties to boost both the quality and quantity of the immunological reaction against vaccine antigens. Immunopotentiators are increasingly being used in adjuvants, however if they are not properly designed to manage these effects, their usage may result in increased adverse outcomes. Freely soluble immune potentiators are particularly challenging because they can diffuse quickly from the administration site into systemic circulation, potentially causing severe adverse effects.[9] To address these issues, various nanocarrier approaches, such as polymeric nanoparticles, emulsions, liposomes, and insoluble aluminum salts, have been proposed to enhance immunopotentiator pharmacokinetics and biodistribution characteristics, as well as stability, toxicity, and target delivery.

1. Of lipid-Based Formulations: Discovery, Characteristics, and Basics Manufacturing

Liposomes were discovered by Alec D. Bangham in the 1960s at the Babraham Institute, University of Cambridge. They consist of biocompatible and biodegradable phospholipid bilayers. Amphipathic molecules with a hydrophilic head and a lipophilic (hydrophobic) tail are known as phospholipids. A bilayer structure is formed during liposome production when the hydrophilic heads align with the aqueous medium and the hydrophobic tails form the inner part of the membrane.[10]

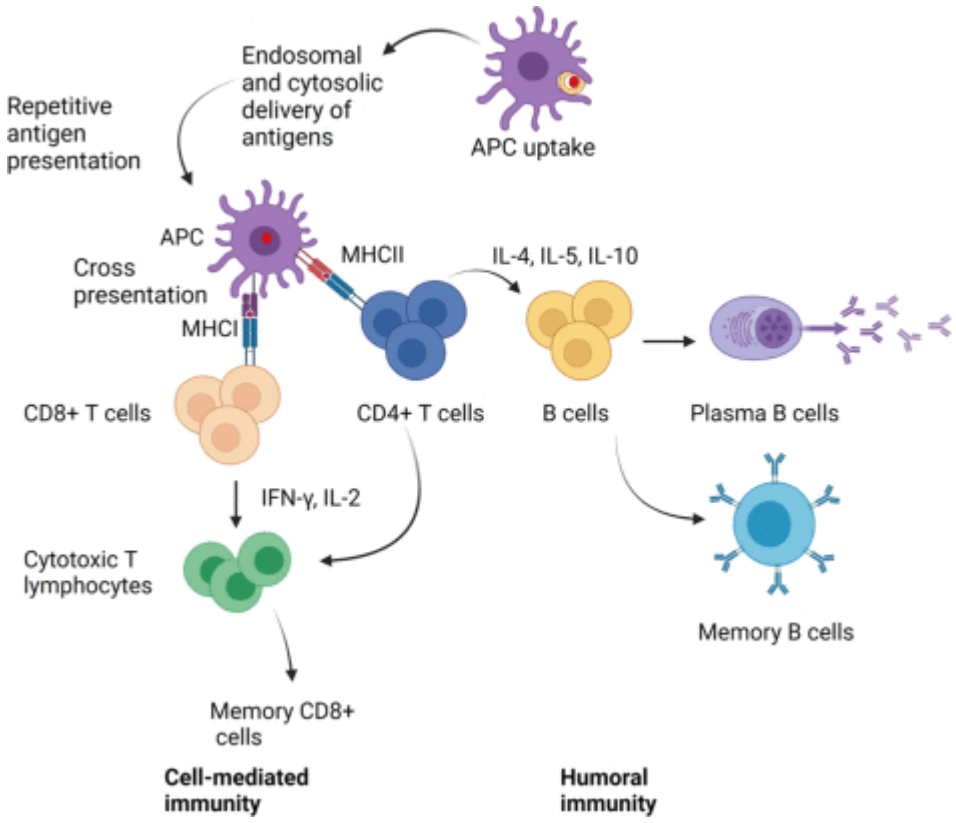
According to their shape, liposomes are often divided into two main categories: unilamellar and multilamellar vesicles.

- ***Unilamellar Liposomes (ULVs)****:* Formed by a single bilayer of phospholipids surrounding an aqueous core.
  - ***Small Unilamellar Vesicles (SUVs):*** Less than 100 nm in size.
  - ***Large Unilamellar Vesicles*** **(LUVs)**: Can be up to a few micrometers in size.
- ***Multilamellar Liposomes (MLVs)****:* Consist of several concentric bilayers separated by aqueous compartments. Factors such as the number of bilayers, lipid composition, and manufacturing method influence the size of the liposomes.[11]

Nowadays there is a plethora of lipid combinations that can be considered for the development of lipid-based formulations. Neutral phospholipids like 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), l-α-phosphatidylcholine (HSPC), and 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) are common components of liposomes and LNPs. They provide the structures of lipid bilayers and cholesterol, which improve membrane stability. The structure and surface characteristics of liposomes can be altered by using negatively (1,2-dioleoyl-sn-glycero-3-phosphate (DOPA), 1,2-dioleoyl-sn-glycero-3-phospho-l-serine (DOPS)) or positively (1,2-dioleoyl-3-trimethylammoniumpropane (DOTAP)) charged lipids. Due to the presence of surface charge, which creates electrostatic repulsion to stop flocculation and aggregation, charged liposomes often exhibit great stability. Ionizable cationic lipids (such DLinDMA) and PEG lipid are essential parts of LNPs because they provide high RNA encapsulation efficiencies and the steric barrier effect, respectively. Ionizable cationic lipids have been introduced in the early 2000s, and they have the ability to change their pH according to the pH of the environment. Thus, they have a positive charge at acidic pH and become neutral at physiological PH.[12]

2. Liposomes as Vaccine Based Adjuvants

Currently, approved vaccines contain three different kinds of adjuvants: liposomes, oil-in-water emulsions, and aluminum salts. The ability of liposomes to effectively transport hydrophilic and hydrophobic molecules, including immunoproteins and antigens, is one of their main advantages over the others. The physicochemical (size and charge) and immunogenic (incorporation of additional adjuvants and targeting ligands) characteristics of liposome vaccine adjuvants determine their capacity to draw in, engage with, and activate APCs (e.g., dendritic cells (DCs), macrophages, and B cells). For example, cationic liposomes' positively charged surface encourages interactions with DCs' negatively charged surface, which makes antigen absorption and distribution easier. Major Histocompatibility Complex (MHC)-mediated antigen presentation to T cell receptors (TCRs) makes DCs one of the primary inducers of T cell-mediated immune responses, making them extremely significant. With the right targeting ligands, modified liposomes may stimulate and activate cells through PRRs, which leads to antigen processing and presentation as well as the maturation of APCs (co-stimulatory molecules and cytokine signals). To make sure that T and B cells react to the infection in the right way, these signals from APCs control their polarization.[13]



Induction of humoral cellular immunity by liposomal delivery. Liposomes are taken up by immature APCs and present antigen by MHCII to naïve CD4+ T cells. CD4+ T cells become activated and proliferate to Th1 and Th2 subtypes. CD4+ T cells activate B cells through IL-4, IL-5, and IL-10 to produce antibodies against the antigen. Antigens can also be found in the cytosol of DC, which allows them to be presented by MHC I, directly activating cytotoxic T lymphocytes. In this case, Th1 cells produce IFN-γ and IL-2, which favor cellular activation and hence cytotoxic T cell.

3. Combinatoral Adjuvant Statergies

Adjuvants can be derived from many types of molecules including vitamins, carbohydrates, peptides, proteins, antibodies, aptamers, and enzymes.[14][15] Because they affect the system's efficiency, the loading mode and the liposomes' physicochemical characteristics such as their size, fluidity, and surface charge—are crucial.[16] The type of molecules, the physicochemical characteristics of liposomes, and the system's ultimate use all influence the strategy that should be used. Nanoscale multifunctional liposomal formulations, such as easy coadministration, encapsulation, Surface functionalization and modification techniques have been used to generate surface decoration via electrostatic complexation or covalent bonding.[17]

Antigens, adjuvants, and targeting ligands can be incorporated into or onto liposomes using a variety of techniques.

A) Encapsulation: During production, hydrophilic substances including proteins, antigens, DNA, and RNA can be incorporated into the liposomes' watery core. It is possible to integrate hydrophobic compounds into the lipid bilayer.

B) Electrostatic Binding: Different targeting moieties and antigens can electrostatically bind to the oppositely charged lipids on the liposome surface.

Conjugation: Targeting ligands, including proteins, peptides, antibodies, and small molecules, can be covalently attached to functionalized lipid anchors on the surface of liposomes to provide targeted distribution.

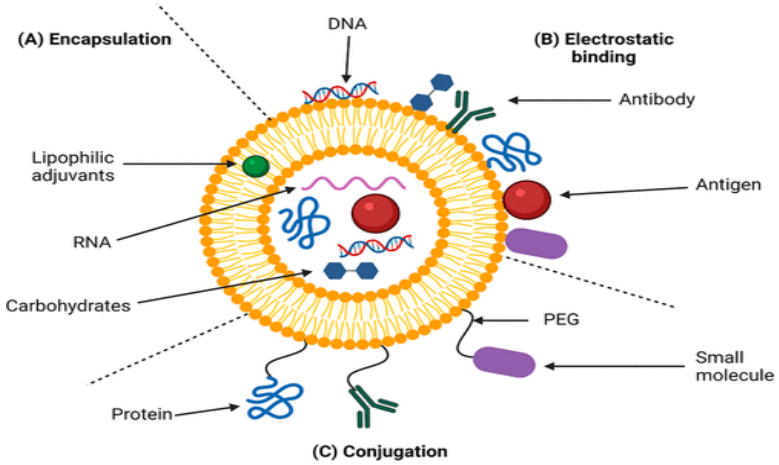


Figure no 3: Methods for adding targeted ligands, adjuvants, and antigens to or onto liposomes.

4. Coadministration of Lipid-Based Nanoparticles, Antigens, and Other Adjuvants

When compared to other methods, a straightforward physical combination of antigen and manufactured liposomes is far more practical. Even if there are worries that adjuvants and antigens may dissociate once they enter the body if they are simply mixed, [18] leading to rapid degradation.[19][20] In many cases, this strategy has worked well, particularly with promoted products. This method has been used in several human vaccinations that comprise a combination of protein antigens suspended in aluminum hydroxide or aluminum phosphate.[21] Following mixing, the aluminum quickly interacted with different antigen groups, causing them to associate and adsorb. The Mosquirix and Shingrix vaccines, which prevent shingles and malaria, respectively, employ the simple mixing technique in which the AS01 adjuvant is combined before injection.

5. Encapsulation

All molecules can be encapsulated into liposomes, regardless of how hydrophobic they are. However, the physicochemical characteristics of the molecules in issue (polarity, partition coefficient) have a significant influence on the encapsulation technique and degree. Hydrophobic compounds can be easily incorporated into liposomes during the manufacturing process, with high encapsulation efficiency.[22] Because these compounds are lipophilic, they may be added to the dissolved lipid organic phase and directly incorporated into the lipid bilayer. However, throughout the production process, hydrophilic substances including peptides, antigens, and nucleic acids can be introduced to the hydrophilic core of liposomes. The molecule to be entrapped may be present in the lipid film (for lipophilic molecules) or the aqueous medium (for hydrophilic molecules) when the thin film hydration technique is applied. Lipids can dissolve in the organic phase and hydrophilic adjuvants in the aqueous phase of a microfluidic device. During the manufacturing process, hydrophilic chemicals are injected into liposomes with great encapsulation efficiency after both streams are simultaneously injected into the chip.

6. Surface modification

Adjuvants can be added to the surface of liposomes by covalently attaching to PEG or lipid anchors or by electrostatically interacting with them. Optimizing the ligand density on the liposome surface is a crucial step in customizing a liposomal system. Prior research has demonstrated that a higher ligand density on the particle surface increases cellular uptake.[23–26] However, going above an ideal ligand density might lead to problems including aggregation and a diminished therapeutic efficacy.[27]

7. Electrostatic Binding

The liposomes and adjuvants/antigens must have opposing electric charges in order for surface integration to occur by electrostatic binding. The degree of adsorption is determined by the intensity of the electrostatic interactions that exist between them. The protein antigens adsorb onto cationic liposomes at physiological pH because they are negatively charged and have isoelectric points (pIs) less than 7.4.Compared to neutral and anionic liposomes, cationic liposomes have been shown to elicit a greater immunogenicity. According to reports, chemokine induction plays a role in cationic liposomes' potent adjuvanticity. When DC was stimulated, Yan et al.'s comparison of neutral, negative, and cationic charged liposomes showed that only cationic liposomes could up-regulate the CCL2 chemokine gene. This induction was mediated by the ERK pathway both in vitro and in vivo. [28,29]

8. Covalent bonding

On the surface of liposomes, adjuvants can also be covalently bonded. Three types of reactions can be used to functionalize molecules with different targeting ligands: amide bonds are formed between carboxyl and amino groups; disulfide bonds are formed by the reaction of pyridyldithiols and thiol groups; and thioether bonds are formed by the reaction of maleimide and thiol groups.[30]

It has been shown that the creation of sophisticated lipid-based formulations depends on the glycosylation of liposomes by the insertion of carbohydrates (such as mannose or glucose). Different glycoproteins and glycolipid receptors found in many human cells are able to identify bacterial glycans, such as mannose, on the cell walls of infectious organisms (viruses, fungi, bacteria, etc.).[31][32] When included in the liposome formulation, carbohydrate-based structures have the potential to interact with lectins. Drug delivery systems have been designed by taking advantage of the interactions between carbohydrates and lectins.[33][34]

Table no 1: Advantages and Disadvantages Incorporation methods.

Strategy	advantages	disadvantages
Coadministration	Simplicity	Rapid degradation
Encapsulation	High encapsulation efficiencies for lipophilic molecules	Low encapsulation efficiencies for hydrophilic molecules
	Protection from degradation	Not suitable for high MW antigens
	Co-delivery can be ensured	Opposite electric charges are required.
Electrostatic binding	Simplicity	Exposure of nucleic acid– based adjuvants to nucleases.

LIPOSOMES FOR POLYSACCHARIDE DELIVERY

Liposomal Glycoconjugate Vaccines

Liposomes have emerged as promising carriers for glycoconjugate vaccines, combining polysaccharide and protein antigens to enhance immunogenicity.[35] Here's an overview of recent advancements and applications in this field:

Dual-Functioning Liposomal Formulations

1. ***Jones et al.*** proposed a novel approach where polysaccharides from Streptococcus pneumoniae are encapsulated within liposomes, while protein antigens are surface-exposed on the liposomal surface. This design allows for noncovalent colocalization of polysaccharide and protein antigens, mimicking the glycoconjugate structure. This method simplifies production and potentially offers broader serotype coverage for pneumococcal vaccines.[36]

2. ***Co-Delivery of Polysaccharides and Proteins****:*

- ***Li et al.*** utilized liposomes to co-deliver 20 different Streptococcus pneumoniae polysaccharides along with two pneumococcal protein antigens (GlpO and PncO). Protein antigens were surface-decorated using a modified lipid component (DOGS-NTA-Ni), which allowed for His-tagged antigen attachment. Polysaccharides were encapsulated within the liposomes. This approach demonstrated broad vaccine coverage against 70 serotypes of Streptococcus pneumoniae, including those not covered by existing vaccines.[37]

3. ***Next-Generation Glycoconjugate-like Vaccines***

- Continuing their work, Li et al. developed a next-generation pneumococcal vaccine comprising 24 serotypes encapsulated in liposomes and surface-engineered solely with the PncO protein antigen. This formulation elicited significant capsular polysaccharide (CPS) antibody titers across all serotypes, comparable to licensed pneumococcal vaccines PCV13 and PPSV23. They also explored alternative noncovalent attachment methods, such as streptavidin-biotin-based strategies, demonstrating the adaptability of their system.[38]

Applications and Immunological Responses

- ***Protection Against Streptococcus pneumoniae***: These liposomal glycoconjugate Vaccines have proven to be effective in animal models against various serotypes of Streptococcus pneumoniae, highlighting their potential to offer extensive resistance against pneumococcal infections, including those resistant to current vaccines.[39]
- ***Diepitope Constructs for Shigella flexneri****:* Said Hassane et al**.** synthesized oligosaccharides that resemble the influenza hemagglutinin peptide HA 307-319 (Th epitope) and the O-antigen of Shigella flexneri 2a lipopolysaccharide (B-cell epitope) in liposome constructions. These constructs were decorated with both B and T-cell epitopes via coupling to the TLR2 agonist Pam3CAG, incorporated into liposomes during preparation. In

mouse models, these synthetic liposomes induced antibody responses against native lipopolysaccharide and protected against Shigella flexneri 2a challenge.[40]

LIPID-BASED PARTICLES FOR MRNA DELIVERY

mRNA-Based Vaccines and Lipid-Based Delivery Systems

In recent years, mRNA-based therapeutics have emerged as a highly promising class of biologics, particularly in the field of vaccines and cancer immunotherapy. RNA technology allows for the in vivo expression of proteins, inducing robust humoral and cytotoxic T cell responses. To overcome RNA's natural instability, various lipid-based nanocarriers have been developed for its protection and efficient delivery to target sites. Among these carriers, lipid nanoparticles (LNPs) have gained significant attention due to their favorable properties such as small size, serum stability, and efficient encapsulation of RNA.[41][42][43][44]

mRNA Vaccines Development

1. ***Infectious Diseases and Cancer Targets***

- mRNA vaccines have been explored extensively for infectious diseases and cancer. For instance, Bahl et al. used an LNP-formulated mRNA vaccine encoding the HA antigen of influenza H10N8, demonstrating robust antibody responses in clinical studies.
- Moderna and BioNTech have developed multiple mRNA-LNP formulations targeting Zika virus, influenza, human metapneumovirus, human cytomegalovirus, and other pathogens. BioNTech has also advanced liposome-based mRNA vaccines targeting melanoma and breast cancer.[45]

2. ***COVID-19 Vaccines***

- mRNA technology has been pivotal in the rapid development of vaccines against SARS-CoV-2. Moderna's mRNA-1273 and BioNTech/Pfizer's BNT162b2 vaccines, both formulated in LNPs, have been widely successful. These vaccines encode the spike protein or its receptor binding domain (RBD) of SARS-CoV-
- ***Moderna's mRNA-1273****:* Composed of mRNA encoding the spike protein in an LNP formulation.
- ***BioNTech/Pfizer's BNT162b2****:* Utilizes modified nucleoside technology to enhance cell transfection and is also formulated in LNPs.

3. ***Efficacy and Immunogenicity***

- Both mRNA vaccines against COVID-19 have demonstrated over 90% efficacy in clinical trials, providing robust protection against COVID-19.[46]
- Studies on LNP-encapsulated mRNA vaccines targeting SARS-CoV-2 have shown high levels of neutralizing antibodies and significant protection against viral challenge in animal models.[47]

4. ***Advantages of LNPs***

- LNPs containing ionizable cationic lipids are particularly effective due to their ability to encapsulate RNA efficiently and facilitate cellular uptake via endocytosis. These LNPs maintain stability in serum and possess a cationic charge in endosomal pH, enhancing RNA delivery.[48]

mRNA Vaccines

In recent years, several mRNA vaccines entered clinical studies (reviewed elsewhere targeting infectious diseases and cancer, including Bahl et al., who used an LNP-formulated mRNA vaccine encoding the HA antigen of influenza H10N8. All participants developed anti-HA antibody titers after two intramuscular immunizations administered 3 weeks apart.[49] In addition, the study showed that virus-neutralizing antibodies titers of ≥20 were present in the serum of 87% of the vaccinated participants after 43 days of vaccination. Since then, Moderna has taken the lead with several mRNA-LNP formulations being under clinical trials against the Zika virus, influenza, human metapneumovirus (HMPV), human parainfluenza virus type 3 (HPIV3), human cytomegalovirus (HCMV), and chikungunya virus (CHIKV). Regarding cancer immunotherapy, two liposome-based mRNA formulations developed by BioNTech reached the clinic, targeting melanoma and breast cancer.[50]

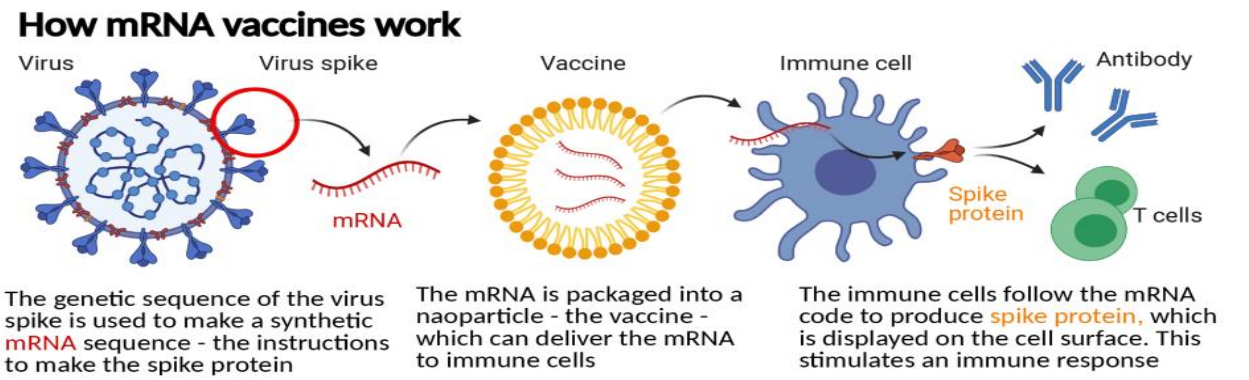


Fig no. 4: m-RNA working.

mRNA has also proven to be highly effective for the development of vaccines against COVID-19. Results from preclinical studies were proven to be highly promising with many formulations reaching the clinics . As a potential vaccine, Zhang and associates created a thermostable LNP-encapsulated mRNA that encodes the receptor binding domain (RBD) of SARS-CoV-2 (ARCoV). According to their immunogenicity investigations, mice given two doses of the ARCoV vaccination developed significant levels of antibodies that had a wide range of neutralizing properties against SARS-CoV-2.[51]Additionally, the ARCoV immunization offered complete defense against the SARSCoV-2 challenge. Lastly, stability tests shown that ARCoV can be kept for at least a week at room temperature. Elia et al. also used RBD conjugated to human Fc for the development of a more stable mRNA-LNP vaccine against SARS-Cov-2 (LNP RBD-hFc mRNA vaccine). *In vivo* evaluation in ACE2 (K18-hACE2) human transgenic mice model showed robust humoral responses comprising binding and neutralizing antibodies, enabling 70% survival of animals administered with a lethal dose of SARS-CoV-2, compared with full mortality in the control group of unvaccinated animals.[52]

In 2020, the first RNA-based vaccines were approved for emergency use by the FDA against COVID-19. mRNA-1273 developed by Moderna was composed of an mRNA vaccine encoding the spike protein of SARS-CoV-2 in an LNP formulation. BNT162b2 was developed by BioNTech in collaboration with Pfizer after evaluation of four different mRNA formats targeting antigens of the spike protein and receptor binding domain and also formulated as an LNP. The compositions of the LNPs of the Pfizer/BioNTech and Moderna mRNA vaccines are similar. Both vaccines are based

on the modified nucleoside technology to increase cell transfection capacity for protein expression and improve tolerability. They demonstrated more than 90% efficacy, providing almost complete protection against COVID-19.[53][54]

Composition of LNPs in Licensed COVID-19 Vaccines[a]

Vaccine	company	composition of LNPs
BNT162b2	BioNTech/Pfizer	DSPC, cholesterol, PEG2000-DMA, ALC-0159, ALC-0315
mRNA-1273	Moderna	DSPC, cholesterol, PEG2000-DMG, SM-102

Table no 2: Composition and companies of LNP's

Along with these vaccines, other mRNA based candidates are under clinical trials and numerous are also at the preclinical stage. Examples are the CVnCoV and LUNAR-COV19/ARCT-021 developed by CureVac and Arcturus Therapeutics/Duke—NUS Medical School, respectively. CVnCoV comprises LNP-formulated, nonchemically modified, sequence engineered mRNA encoding full-length spike protein. CVnCoV is currently being studied in a phase 3 clinical trial. LUNAR-COV19/ARCT-021 is a self-transcribing and replicating RNA (STARR)-based vaccine encoding an alphavirus-based replicon and the SARS-CoV-2 full-length spike glycoprotein. STARR combines self-replicating RNA with LUNAR technology which is a lipid-mediated delivery system. This candidate is currently in phase 2 clinical trials. Although full data related to exact formulation composition of CVnCoV and LUNAR-COV19/ARCT-021 have not yet been disclosed, LNPs consists of DSPC, cholesterol, PEGylated lipid, and a cationic lipid. Preliminary data from both candidates are very promising demonstrating high tolerability and safety profile.

- ***Future Directions***

Lipid-based nanoparticles play an important role in this context as they have intrinsic adjuvant properties. Small compounds or glycans may be readily added to lipid nanoparticles to target the vaccination to subsets of certain immune cells. This increases receptor engagement and boosts the immune response's efficacy. Additionally, lipid nanoparticles can be used to contain adjuvants or antigens with varying physicochemical characteristics. The combination of these characteristics makes this class of particles extremely useful for delivering different vaccine antigens, including as proteins, carbohydrates, and nucleic acids, to fight cancer and infections.

Recent licensing of RNA vaccines against COVID-19 has resulted in a further advancement in the field, since LNPs are key to ensure antigen delivery. This class of vaccine is currently under use mainly in adults but has been clinically validated in adolescents, owing it to be an important weapon among the tools available to fight the ongoing pandemic. Lipid nanoparticles are now being used to transport antibodies in addition to vaccines, which broadens their usage from prevention to therapy.

CONCLUSIONS

Vaccines represent a global priority for the improvement of healthcare worldwide, with adjuvants playing a vital role in further vaccine development. Although big advances have already been made in vaccine adjuvants, there are still important challenges to address. There is a critical need for the development of adjuvants that can break the immune tolerance and induce strong T cell responses for potential use in cancer vaccines. In addition, adjuvants that are able to induce potent immune responses in immunologically hyporesponsive populations, such as the elderly, immunocompromised, and other chronically sick individuals, are required. Combinatorial adjuvant strategies, e.g., AS01, have appeared as a viable approach to overcome these obstacles. Vaccine scientists can now choose between a larger panel of compounds and technologies to design and develop formulations to generate potent immune responses,

suitable for different pathogens, considering that eLipid-based nanoparticles play an important role in this context as they have intrinsic adjuvant properties. Small compounds or glycans may be readily added to lipid nanoparticles to target the vaccination to subsets of certain immune cells. This increases receptor engagement and boosts the immune response's efficacy. Additionally, lipid nanoparticles can be used to contain adjuvants or antigens with varying physicochemical characteristics. The combination of these characteristics makes this class of particles extremely useful for delivering different vaccine antigens, including as proteins, carbohydrates, and nucleic acids, to fight cancer and infections.

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