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Advances and Challenges in mRNA Vaccine Technology

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Abstract: Since the outbreak of COVID-19, mRNA vaccine technology has achieved groundbreaking advancements. Characterized by its high safety profile and potent immune activation capabilities, this technology has demonstrated significant potential in the prevention of infectious diseases and cancer therapeutics, marking a new milestone in vaccine development. This review focuses on three key aspects—molecular design, delivery systems, and immunological mechanisms—providing a comprehensive analysis of structural optimization strategies, delivery methodologies, and immune modulation approaches for mRNA vaccines. Additionally, it summarizes and evaluates potential challenges that may arise in the future development of mRNA vaccines. By analyzing existing technological pathways, this review aims to advance innovation in mRNA vaccine technology and facilitate its broad applications in public health and veterinary medicine.

Keywords: mRNA vaccine; Molecular design; Delivery systems; Immune mechanisms

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1. Introduction

Vaccines, as core biological agents in modern infectious disease prevention and control systems, have undergone iterative breakthroughs in development technologies such as inactivated, attenuated, recombinant protein, and gene-editing platforms. Through precise immune response mechanisms, these advancements have not only consigned virulent infectious diseases like smallpox to history but also continuously rewritten humanity's offensive and defensive strategies against pathogens. The novel coronavirus pneumonia (COVID-19) pandemic that broke out at the end of 2019 posed unprecedented challenges to global public health and underscored the urgency of vaccine development [1].

Unlike traditional vaccines, mRNA vaccines do not contain live pathogens or proteins. Instead, they utilize delivery systems to transport pathogen genetic information into host cells, providing instructions for intracellular processing and production of virus-related antigenic proteins, thereby activating the body's immune response [2].

Building on these unique modular design advantages and rapid response mechanisms, mRNA vaccine technology has broken through traditional R&D paradigms. Its distinctive antigen-coding flexibility and controllable immunogenicity have significantly improved cross-protective efficacy against viral variants, marking the entry of vaccinology into a new stage of precise regulation.

During the COVID-19 pandemic, Pfizer-BioNTech and Moderna successively announced clinical trial results for their COVID-19 mRNA vaccines. Data from phase 3 clinical trials showed that both vaccines achieved protective efficacy rates exceeding 94%, with a low incidence of severe adverse events. They were approved for marketing and use in the United States in December 2020 [3]. The successful application of mRNA vaccines has not only provided a powerful tool for pandemic control but also pointed out new directions for the development of vaccines against other infectious diseases. This article will review the molecular design, delivery systems, immune mechanisms, and challenges of mRNA vaccines.

2. Classification and molecular design of mRNA vaccines

mRNA vaccines are classified into non-replicating mRNA (NRM) vaccines, self-amplifying mRNA (SAM) vaccines, and circular RNA (circRNA) vaccines based on their genetic characteristics (**Figure 1**).

2.1. Non-replicating mRNA vaccine

NRM vaccines are currently the most widely used mRNA vaccine type in clinical applications. Their design is based on the structural framework of natural mRNA, including the 5' cap, untranslated region (UTR), open reading frame (ORF), and poly(A) tail. The optimized combination of these structural elements significantly enhances the stability, translation efficiency, and immunogenicity of mRNA.

2.1.1. 5' cap

The 5' cap is critical for mRNA stability and translation initiation. Common cap structures include cap 0 (m7GpppNp), cap 1 (m7GpppNmp), and cap 2 (m7GpppNmpNmp) [4]. Among these, cap 1, featuring 2'-O-methylation modification, effectively avoids recognition by the host innate immune system and has become the preferred choice for mainstream vaccines [4]. Capping methods are divided into enzymatic capping and co-transcriptional capping: enzymatic capping involves multi-step enzymatic reactions, while co-transcriptional capping uses cap analogs to directly generate cap 1 structures during *in vitro* transcription. Moderna's mRNA-1273 employs enzymatic capping, whereas Pfizer's BNT162b1 achieves high-efficiency capping via co-transcriptional methods [5,6].

2.1.2. Untranslated regions

The UTR optimization is another core strategy to improve mRNA performance. The 5' UTR enhances ribosome binding efficiency through the introduction of Kozak sequences, while the 3' UTR prolongs mRNA half-life by removing AU-rich degradation elements [7,8]. Additionally, UTR sequences from naturally highly expressed genes such as human hemoglobin α/β subunits (HBA/HBB), albumin (ALB), or heat shock proteins (Hsp70) can be used as direct substitutes [9].

2.1.3. Open reading frame

The ORF is the target antigen-coding region. Selecting appropriate optimization strategies in this region can enhance overall mRNA translation efficiency. Replacing uridine with chemically modified nucleotides such as pseudouridine or N1-methylpseudouridine significantly reduces mRNA immunogenicity while increasing resistance to RNases [10]. Furthermore, rare codons in the ORF can be replaced based on the degenerate codon preferences of different hosts to improve translation efficiency [11].

2.1.4. Polyadenylate tail

The poly(A) tail synergizes with the 5' end-cap structure to inhibit the degradation and stability of mRNA by exonucleases. The optimal poly(A) tail length varies by cell type, with studies suggesting a range of 120–150 nucleotides [12,13]. BioNTech's segmented poly(A) tail design (A30LA70), incorporating a UGC linker, further extends mRNA retention time within cells [14].

2.2. Self-amplifying mRNA vaccine

SAM vaccines are structurally similar to NRM vaccines but additionally encode viral RNA replication machinery-related genes, enabling self-amplification in host cells to induce high-level antigen expression at extremely low doses.

The core advantage of SAM vaccines lies in their "self-adjuvant effect." After entering the cytoplasm, the SAM-encoded replicase is first translated and assembled into a multi-enzyme complex, which replicates the input mRNA into negative-strand RNA and subsequently generates new genomic mRNA and subgenomic mRNA. The latter drives efficient antigen protein expression via subgenomic promoters while activating strong innate immune responses (i.e., the "self-adjuvant effect"). For example, the LNP-nCoVsaRNA vaccine developed by Imperial College London, based on Venezuelan equine encephalitis virus (VEEV) genes, requires only 0.1–10 μg to induce high-titer neutralizing antibodies in mice [15].

However, the long sequences (typically exceeding 9 kb) and complex secondary structures of SAM vaccines pose production challenges. To address this, researchers have developed trans-amplifying RNA (taRNA) vaccines by separating the replicase gene and antigen gene into two distinct RNA molecules [16,17]. This dual-vector system not only simplifies production but also minimizes replication-induced interference with host cells. For instance, in influenza vaccine studies, 0.05 μg taRNA achieved complete protection in mice, demonstrating ultra-high dose efficiency [17].

2.3. Circular RNA vaccine

CircRNA vaccines are an emerging form of mRNA vaccines characterized by a covalently closed circular structure. Unlike linear mRNA, circRNA resists exonuclease degradation without requiring a 5' cap or poly(A) tail, enabling prolonged intracellular persistence. Its closed structure also evades recognition by pattern recognition receptors, reducing innate immune responses and dependence on nucleotide modifications [18]. However, circRNA translation efficiency is limited by the activity of internal ribosome entry sites (IRES). Studies show that traditional virus-derived IRES can drive translation but with far lower efficiency compared to the cap-dependent mechanism of linear mRNA [19]. Additionally, the design of long circRNA sequences and residual linear RNA contaminants during production complicate manufacturing processes.

To overcome these challenges, circRNA vaccine optimization focuses on enhancing translation efficiency

and practicality. Research has identified IRES from human rhinovirus B (HRV-B), enterovirus B (EV-B) (e.g., iHRV-B3 and iEV-B107) that exhibit stronger translational activity in circRNA. Inserting synthetic aptamers to enhance interactions with translation initiation factors can significantly boost translation efficiency [19]. Currently, circRNA vaccine development remains in its early stages.

Figure 1. Molecular design of different types of mRNA vaccines

3. Delivery systems for mRNA vaccines

mRNA must cross the cell membrane to enter the cytoplasm to exert its effects. However, due to its large molecular weight and negatively charged nature, mRNA cannot penetrate the cell membrane and is prone to recognition and degradation by the immune system. Therefore, it is very necessary to develop efficient mRNA vaccine delivery systems. To date, scientists have achieved significant progress in mRNA vaccine delivery systems, which can be broadly classified into two categories: physical delivery and carrier-assisted delivery methods (**Figure 2**).

3.1. Physical delivery of naked mRNA

Physical delivery methods overcome the cell membrane barrier through external forces to directly introduce exogenous mRNA into target cells or tissues. These primarily include direct injection, electroporation, and gene gun technologies.

3.1.1. Direct injection

Direct injection of mRNA is commonly used in cancer therapy, with major administration routes including intramuscular, subcutaneous, intradermal, and intralymphatic approaches. In 2013, Phua *et al.* [20] found that subcutaneous injection of naked mRNA in mice achieved higher delivery efficiency than mRNA nanoparticle delivery methods. Van Lint *et al.* [21] proposed that intertumoral injection of tumor-associated mRNA triggers appropriate immune responses and could become a promising vaccination strategy. Recently, more and more researchers have focused on the role of naked mRNA in treating or preventing infectious diseases. The team

of Abbasi [22] employed a needle-free jet injector (PYRO) that utilizes transient liquid pressure to promote the internalization of naked mRNA into skin cells. This method induced local lymphocyte infiltration, significantly reduced viral load in challenged mice, alleviated tissue damage, and provided effective immune protection.

3.1.2. Electroporation

Electroporation is one of the most widely used physical delivery techniques. Its principle involves applying a brief high-voltage electric field to create transient pores in the cell membrane, facilitating mRNA entry into cells. Since its first application in gene transfection in 1982, electroporation has been commonly used for *in vitro* mRNA transfection of hematopoietic cells [23]. In tumor immunotherapy, electroporation is employed for dendritic cell (DC) mRNA transfection, activating T-cell immune responses through tumor antigen-encoding mRNA. For example, clinical trials in melanoma patients demonstrated that electroporation-delivered mRNA induces robust $CD4^+$/$CD8^+$ T-cell responses [24]. Additionally, electroporation exhibits adjuvant effects by recruiting pro-inflammatory cells and inducing cytokine secretion, thereby enhancing mRNA immunogenicity [25]. However, limitations include potential cell membrane damage or apoptosis, and its superior immune-enhancing effects in SAM (self-amplifying mRNA) over NRM (non-replicating mRNA) *in vivo* applications restrict its broader use [26].

3.1.3. Gene gun technology

Gene gun technology uses compressed gas (helium or nitrogen) to propel gold-coated mRNA particles at high speed into target tissues, achieving delivery through physical penetration. Studies have confirmed that gene guns can deliver human α-1 antitrypsin mRNA to mouse skin and elicit antibody responses [27]. Subsequent developments applied this technology to mRNA repair therapies for genetic skin diseases, successfully targeting deeper skin layers in mice [28]. Although highly efficient in murine models, its efficacy in large animals and humans remains unverified. Moreover, the physical impact of gold particles may disrupt normal cellular physiology or cause local tissue damage, limiting its clinical translation potential.

In general, the core advantage of physical delivery technology is to bypass vector dependence and directly realize the intracellular delivery of mRNA. In addition, some methods (e.g., electroporation) also function as immune adjuvants. However, its drawbacks should not be ignored: on the one hand, physical external forces may cause cell damage or death, affecting the safety of treatment; On the other hand, technologies such as gene guns are inefficient in the transformation of large animals and humans, and it is difficult to meet clinical needs. In addition, the positioning accuracy of physical methods on target tissues is limited, and it is difficult to achieve systemic delivery. These limitations have prompted research to move to safer, controlled delivery systems such as lipid or polymer nanoparticles. Nevertheless, physical delivery still has irreplaceable value in specific scenarios, such as local tumor therapy or skin targeting, and its efficacy and safety need to be further improved through technological improvements in the future.

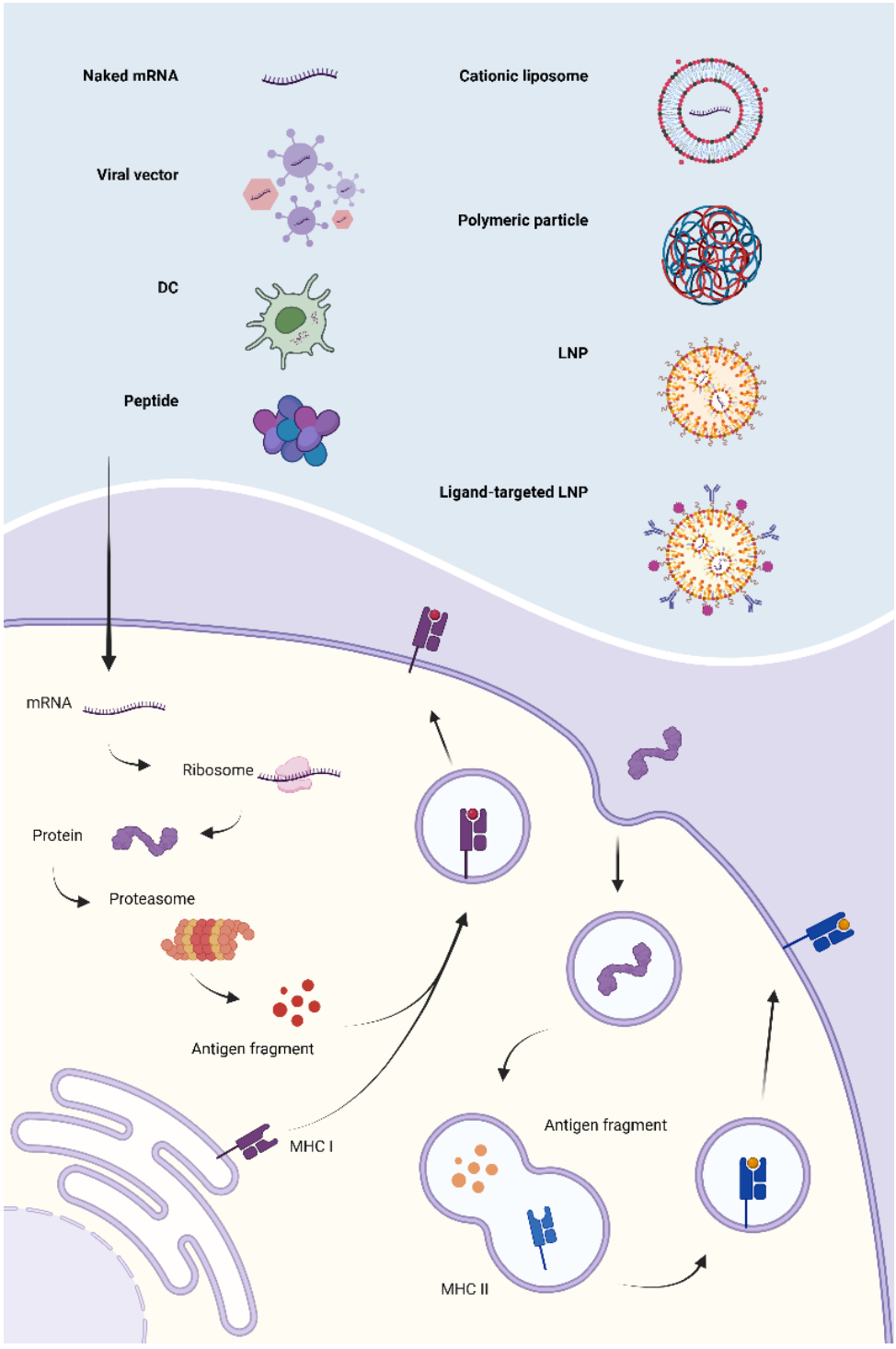

Figure 2. mRNA vaccine delivery mechanisms and adaptive immune response mechanisms

3.2. Carrier-based mRNA delivery methods

Despite the direct intracellular delivery provided by physical methods, their inherent limitations have driven researchers toward more biocompatible carrier systems. Compared to physical interventions, carrier delivery employs biomimetic or engineered designs to mimic natural cellular interaction mechanisms, enhancing mRNA stability and enabling precise delivery. Current carrier systems are divided into biological and non-biological

categories: the former utilizes viral or cellular bioactive units, while the latter relies on synthetic nanomaterials. Below, we systematically elaborate on carrier-based mRNA vaccine delivery technologies and their clinical potential, focusing on breakthroughs in immune activation efficiency, tissue specificity, and safety.

3.2.1. Biological carriers

3.2.1.1. Viral vector

Viral vectors have long been used for RNA drug delivery. Retroviral vectors, among the earliest developed, remain the preferred choice for *ex vivo* transfection of hematopoietic stem cells. Adeno-associated virus (AAV) vectors are widely used in *in vivo* studies due to their safety, broad host cell range, and prolonged transgene expression, making them one of the most common gene therapy vectors [29]. Lentiviral vectors can infect both dividing and non-dividing cells, accommodate inserts up to 8 kb, and enable long-term expression [30]. Studies show that viral vector-delivered mRNA formulations are effective against viral and bacterial diseases and cancer [31]. However, risks such as genomic integration, uncontrollable gene expression, and severe immune side effects limit their clinical adoption.

3.2.1.2. Dendritic cell

DCs are the most potent antigen-presenting cells (APC). After capturing antigens in tissues, they migrate to lymphoid organs to present processed antigens to immune cells, initiating cellular and humoral immunity. The DC-mRNA delivery approach involves transfecting DCs *ex vivo* with antigen-encoding mRNA and reinfusing them into the host to activate specific immune responses. DC-mRNA systems are favored in clinical research for their high delivery efficiency without additional carriers and their strong induction of cellular immunity, particularly in cancer therapy [32]. However, complex and costly production processes hinder large-scale application. Additionally, immune responses triggered during *ex vivo* mRNA transfection may diminish during preparation, reducing therapeutic efficacy [33]. These issues necessitate further optimization for clinical use.

3.2.2. Non-biological carriers

3.2.2.1. Cationic lipids

Cationic lipids were the first-generation lipids developed for mRNA vaccine delivery. Their inherent positive charge enables spontaneous complexation with negatively charged mRNA and attraction to cell membranes. N-[1-(2,3-dioleyloxy)propyl]-N,N,N-trimethylammonium (DOTMA) and its synthetic analog 1,2-dioleoyl-3-trimethylammonium propane (DOTAP) were among the first cationic lipids used for mRNA delivery. Lipofectin, a cationic liposome formulation developed from these, demonstrated strong cell transfection capabilities [34].

However, the cationic nature may lead to nonspecific interactions with negatively charged serum proteins, forming aggregates that shorten half-life and cause adverse effects [35]. To address this, second-generation ionizable lipids were developed. Structurally optimized with tertiary amine groups, these lipids remain neutral at physiological pH but protonate to become positively charged in acidic environments. This pH sensitivity allows these lipids to better circumvent strong interactions with cell membranes during circulation *in vivo*, which in turn significantly reduces their immunogenicity and biotoxicity and improves delivery efficiency [36].

3.2.2.2. Polymers

Polymers can be divided into cationic and anionic polymers, and positively charged cationic polymers are widely

used for mRNA delivery. Polyethylenimine (PEI) is the most researched and commercialized cationic polymer in the field of mRNA delivery, consisting of linear or branched polymers with different relative molecular weights and structures [37]. Due to its intrinsic cationic properties, PEI binds tightly to mRNA through electrostatic interactions, efficiently delivering it into the cell, and releasing mRNA within the cytoplasm through the cleavage of disulfide bonds. However, high positive charge density induces cytotoxicity [38]. To address this challenge, researchers have found that the addition of chemical modifications can significantly improve the delivery and biological tolerance of PEI *in vivo* [39]. In addition, a variety of amine-containing polyester polymers have been developed for mRNA delivery. Polyester-based polymers can significantly improve biodegradability and biocompatibility by introducing labile chemical bonds, including carbonate, ester, amide, and phosphate bonds [40].

3.2.2.3. Peptides

Due to electrostatic action, negatively charged mRNA is easily adsorbed by positively charged peptides for delivery. Protamine is a small molecular weight protein rich in arginine that binds tightly to mRNA to form a stable complex, protects mRNA from RNase degradation in serum, and acts as an adjuvant to elicit a strong immune response [41]. In recent years, protamine delivery platforms have been developed for clinical applications in various vaccines and cancers, showing great prospects for development [42,43].

Cation cell-penetrating peptide (CPP) is another small molecule peptide with excellent delivery ability, low charge density, and cell membrane disrupting properties, which can effectively enhance the endosomal escape efficiency of mRNA [44]. The commercial peptide PepFect14 has been shown to be effective in delivering therapeutic mRNA to ovarian cancer cells in mouse models [45]. Although CPP has shown great potential in the field of vaccine delivery, the US Food and Drug Administration (FDA) has not approved any CPP-conjugated compounds for clinical use due to their lack of tissue specificity and cytotoxicity [46].

3.2.2.4. Lipid nanoparticles

Lipid nanoparticles (LNPs) are currently the most advanced and widely used mRNA delivery system and are the only drug delivery system that has been clinically proven effective and approved for human use [47]. LNPs are nanoscale vesicles capable of mimicking the lipid structure of cell membranes to encapsulate mRNA in their cavities. LNPs are composed of four components: ionizable lipids, pegylated lipids (PEGs), cholesterol, and auxiliary phospholipids. The self-positive charge of ionizable lipids facilitates the self-encapsulation of mRNA and facilitates the escape of mRNA from endosomes upon entry into cells. Hydrophilic PEG can improve the colloidal stability of LNP in biological fluids and prolong the half-life of LNP. Cholesterol and phospholipids primarily support the structural stability of LNP particles [48].

Previous studies have shown that LNPs can greatly promote mRNA delivery *in vivo* and enhance antigen expression, resulting in durable protective immune responses against a variety of infectious agents [49,50]. Companies such as Moderna, Pfizer, and BioNTech have used LNPs in the development of COVID-19 mRNA vaccines against the novel coronavirus (SARS-CoV-2). Many other studies have also validated the efficacy of LNP-mRNA in cancer therapy [51,52]. Although LNP-mRNA vaccines have the advantages of good biosafety, mature industrial production technology, and convenient large-scale production, there are still problems in clinical application, such as low cell uptake rate and prone to inflammatory reactions. Studies have shown that the delivery efficiency of LNPs can be improved by adjusting or modifying the lipid components of liposomes and selecting the appropriate route of administration [53].

3.2.2.5. Targeted delivery

LNPs bind to apolipoprotein E (APOE) and target APOE receptors on the surface of hepatocytes, resulting in most systemically administered LNP-mRNAs targeting only the liver [54]. Therefore, the specific delivery of LNP-mRNA to specific organs and cells has become a major technical challenge. Targeted delivery technology enables precise delivery of mRNA to target cells and tissues by integrating antibodies or aptamers in nanoparticles [55]. This method uses the high-affinity interaction between antigen-antibody or aptamer-receptor to improve the specificity and efficiency of mRNA delivery and reduce the possibility of causing excessive inflammation, which is currently a research hotspot in the field of mRNA drug delivery.

4. Immune mechanisms of mRNA vaccines

The immune mechanism of mRNA vaccines involves the activation of innate and adaptive immunity, as well as the dynamic presentation process of antigens *in vivo*. At its core, antigenic proteins are synthesized using the host cell's translation system and elicit specific protective responses against pathogens through multi-level immune signaling.

4.1. Activation and regulation of innate immunity

Innate immunity is the body's first line of defense against non-self-infused substances, and after exogenous mRNA enters the host cell, pathogen-related molecular patterns in its molecular structure can be recognized by a variety of pattern recognition receptors. For example, single-stranded RNA (ssRNA) is recognized by Toll-like receptors in endosomes (eg, TLR7, TLR8), while double-stranded RNA (dsRNA) contaminants are recognized by RIG-I-like receptors (eg, RIG-I, MDA5) in the cytoplasm or TLR3 in endosomes [56]. The binding of these receptors triggers a downstream signaling cascade that ultimately promotes the secretion of type I interferons and pro-inflammatory cytokines.

Type I interferon has a dual role in immune activation: on the one hand, it lays the foundation for the initiation of adaptive immunity by promoting the maturation of DCs and macrophages, enhancing antigen presentation ability; On the other hand, excess interferon inhibits the translation mechanism of host cells through the JAK-STAT signaling pathway, resulting in reduced antigen expression efficiency of mRNA vaccines [57]. Therefore, balancing the degree of activation of innate immunity is key to optimizing mRNA vaccines.

In order to reduce the negative impact of excessive immune activation on vaccine efficacy, nucleotide modification and novel purification techniques are widely used as feasible methods to improve the efficacy of mRNA vaccines [58,59]. The modified mRNA can significantly reduce the recognition ability of TLR7 and TLR8, reduce the secretion of type I interferon, and improve the stability and translation efficiency of mRNA. Purification processes such as high-performance liquid chromatography (HPLC) can remove dsRNA contaminants from *in vitro* transcribed mRNA, which can avoid abnormal activation of RIG-I and MDA5 and further balance immune stimulation and antigen expression.

4.2. Initiation and amplification of adaptive immunity

After being delivered to the host cell by a vector, the mRNA vaccine escapes from the endosomal to the cytoplasm and is translated by the ribosome into the target antigen protein. A part of the antigenic protein is degraded into

short peptides by the proteasome in the cytosol, which binds to MHC class I molecules and presents them to the cell surface, activating $CD8^+$ T cells and initiating cellular immunity. The other part is secreted extracellular, engulfed by APC as an exogenous antigen, and after being degraded by lysosomes, it binds to MHC class II molecules to activate $CD4^+$ T cells and initiate humoral immunity (**Figure 2**).

Notably, mRNA vaccines have the unique advantage of being able to directly transfect antigen-presenting cells, thereby achieving cross-presentation. For example, DCs can present antigens to $CD8^+$ T cells via the MHC class I pathway after ingestion and translation of mRNA, while activating $CD4^+$ T cells via the MHC class II pathway. This dual-pathway activation mechanism significantly enhances the synergistic effect of T cells, which not only enhances the breadth and potency of cellular immunity but also provides long-lasting and effective humoral immune protection by maintaining germinal center responses to form long-lived plasma cells and memory B cells [60,61].

5. Current challenges for mRNA vaccines

5.1. Insufficient durability of antibody responses

Whether it can induce durable immune protection after vaccination is one of the core indicators to measure its effectiveness. After mRNA vaccination, the antigen produced is captured by the APC and transferred to the lymph nodes, promoting the formation of germinal centers. In this process, B cells, APCs, and follicular helper T cells (Tfh cells) work together to drive the production of high-affinity neutralizing antibodies [62]. Although preclinical studies [63,64] have shown that mRNA vaccines can induce strong germinal center responses and Tfh cell activation against a variety of pathogens such as human immunodeficiency virus (HIV), Zika virus (ZIKV), SARS-CoV-2, etc., the duration of antibody responses varies significantly depending on antigenic properties. As an example, the Pfizer vaccine BNT162b2 detected a strong germinal center B cell response for at least 12 weeks after vaccination, while the Moderna vaccine mRNA-1273 retained high and high antibody levels for six months, but antibody titers gradually decreased over time [65,66]. In addition, virus mutations may lead to a weakening of antibody-neutralizing efficacy. Studies have shown that cross-neutralizing antibody titers against the B.1.351 and P.1 variants of the novel coronavirus are significantly lower than those of the original strains [67,68]. Therefore, the development and design of mRNA vaccines targeting conserved epitopes may be a key strategy to prolong immune protection.

5.2. Need for safety optimization

Although existing mRNA vaccines have shown good safety in clinical trials and practical applications, there is still a need for further optimization. Dose-dependent adverse effects are a major concern, such as the fact that Moderna's H10N8 influenza vaccine experienced serious adverse events in the 400 microgram dose group, which ultimately led to the adjustment of the maximum dose to 100 micrograms [69]. In addition, the incidence of anaphylaxis with mRNA vaccines was significantly higher than with conventional vaccines, with anaphylaxis occurring at 2.2 and 2.5 per million with the Pfizer-BioNTech and Moderna vaccines, respectively [70]. Researchers hypothesize that these allergic reactions are related to PEG components: anti-PEG antibodies are present in about 40% of the population through daily toiletries, which may induce IgM production and accelerate allergic reactions through T-cell-independent pathways that activate B-cell receptors [71]. In preclinical studies, the presence of anti-PEG IgM in animal serum accelerates the clearance of nanoparticles and leads to a complete loss of efficacy for mRNA therapeutics [71,72]. It can be seen that improving the formulation of vaccine ingredients and reducing PEG

dependence will become the future improvement direction of mRNA vaccines.

5.3. Limitations in accessibility and stability

The requirement for cold-chain storage of mRNA vaccines has severely constrained their rollout in low- and middle-income countries. The Pfizer-BioNTech vaccine needs to be stored at ultra-low temperatures at -70°C, while the Moderna vaccine also needs to be at least -20°C, posing a huge challenge for areas with weak infrastructure. In order to prevent the recurrence of a global pandemic, it is necessary to develop a plan for heat-stabilized dosage forms. Preclinical studies have shown that some mRNA vaccines can be stored at room temperature using sequence optimization or lyophilization [73,74]. If these technologies are validated in clinical trials, they will significantly reduce transportation and storage costs and expand vaccine coverage. In addition, increasing production capacity and reducing production costs are also keys to improving accessibility, especially in the face of new variants that require rapid iteration of vaccines, and the flexibility and scale of production will determine the efficiency of response.

5.4. Unique challenges in veterinary applications

In the field of animal medicine, the promotion of mRNA vaccines faces a series of unique challenges, which to a certain extent delay the industrialization process of this technology in the prevention and control of livestock diseases.

First, the core challenge in vaccine development stems from the deep heterogeneity of immune systems between species. Studies have shown significant species differences in mammalian Toll-like receptor signaling pathways, distribution of antigen-presenting cell subsets, and cytokine regulatory networks [75,76]. As a result, mRNA vaccines developed based on the same pathogen may exhibit very different immune response characteristics in different species. In addition, the choice of adjuvant needs to be carefully weighed: polyinocytidylate can effectively enhance Th1 immunity in porcine models, but may induce an excessive inflammatory response in felines; Although aluminium adjuvant can increase avian antibody titers, it may inhibit the cellular immune response in rodents [77-80]. Therefore, it is important to establish a design framework for species fitness based on systematic vaccinology. Researchers need to integrate multi-dimensional parameters such as epitope prediction of B/T cells of target animals, species-specific codon optimization algorithms, and biocompatibility assessment of delivery systems to achieve accurate cross-species mRNA vaccine development.

Second, the contradiction between economy and large-scale production is particularly prominent. The livestock industry needs low-cost, high-coverage vaccines to prevent and control large-scale outbreaks, and while mRNA technology has the advantage of rapid adaptation to new pathogens, the high standards of plasmid purification and dsRNA contaminant removal in GMP manufacturing can drive up costs. In addition, large-scale farmed animals often need to be vaccinated by non-injection methods such as oral administration and spraying, which puts forward higher requirements for the stability and delivery efficiency of mRNA. Preclinical studies have shown that mRNA encapsulated in LNPs is easily degraded in a simulated gastric acid environment, and how to develop a delivery system that can tolerate the digestive tract environment is an urgent problem to be solved [81,82].

In addition, the duality of regulatory and safety standards also makes the development of mRNA vaccines more difficult. Animal vaccine approvals are often more cost-effective than safe, but consumers may be skeptical about the residual risk of mRNA vaccines in food animals. Although available data suggest that mRNA components can be rapidly degraded in animals, further research is needed on whether lipid carriers (e.g., PEGs)

can be delivered to humans through meat or dairy products [26,83]. Regulators need to establish a cross-species safety assessment framework and promote public education to dispel misconceptions such as "genetically modified animal products."

6. Conclusion

Through molecular design optimization, delivery system innovation, and immune mechanism analysis, mRNA vaccine technology has established its milestone position in the prevention and control of infectious diseases and tumor treatment. The pseudouridine modification and segmented poly(A) tail design of NRM significantly improved the stability, and the low-dose and high-efficiency expression characteristics of SAM provided a new idea for broad-spectrum immunity, while the closed structure of circRNA broke through the restriction of enzyme degradation, but still needed to solve the bottleneck of translation efficiency. In the delivery system, LNP has become the mainstream due to its targeting and industrial maturity, but the problems of PEG-related allergic reactions and hepatic enrichment still need to be broken through. The study of immune mechanisms revealed the core advantages of mRNA vaccines in activating dual-pathway adaptive immunity through cross-presentation, but the challenges of insufficient antibody persistence, viral escape mutations, and cold chain dependence still restrict their application and promotion.

In the future, the development of mRNA vaccines needs to focus on three directions: first, deepen molecular design, develop targeted conserved epitope and species-appropriate sequences, and improve clinical practicability. Second, innovate delivery technology, develop reliable carriers that are resistant to digestion or stable at room temperature, and explore de-PEGylated lipids and organ-specific targeting strategies to reduce the risk of immunogenicity. Third, promote process upgrading, optimize production costs and accessibility through technological innovation, and establish a cross-species safety assessment framework to accelerate the industrialization of veterinary vaccines. At the same time, expanding application scenarios to cancer, gene editing, and rare disease treatment will be the key to technological breakthroughs.

The continuous evolution of mRNA technology will not only reshape the emergency response system for emerging infectious diseases but also lead to a new era of personalized medicine and precision immunotherapy. Through interdisciplinary collaboration to overcome the barriers to stability, safety, and transformation, it is expected to achieve full coverage from human public health to animal health management, providing a solution with both speed and effectiveness to the global health crisis.

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