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Nanostructured lipid-based adjuvants and delivery systems for antiviral vaccine development

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ABSTRACT

Lipid-based vaccines, such as liposomes, emulsions, and lipid nanoparticles, play a key role in the development of antiviral vaccines due to their unique immunomodulatory properties, capacity to serve as antigen depots, ability to protect antigens, and adjuvant effect. Among the various approved vaccines, the vast majority utilize lipid-based adjuvants and delivery systems, such as AS01, AS03, MF59, and BNT162b2. These formulations establish an inflammatory microenvironment at the injection site, recruit immune cells, and activate innate immune pathways, thereby enhancing the immunogenicity of the antigen. Concurrently, they protect antigens from enzymatic degradation and thereby maintain the stability of antigens. This review comprehensively summarizes approved lipid-based antiviral vaccines, encompassing their composition, structure, and underlying immunological mechanisms. Furthermore, it outlines emerging trends in the development of diverse lipid-based vaccine platforms, including strategies to enhance antigen loading capacity, achieve more precise targeting of antigen-presenting cells, and implement novel combinatorial approaches involving multi-platform and multi-pathway strategies. Finally, the review addresses the prevalent challenges, future development directions, industrial-scale manufacturing considerations, and quality control requirements for lipid-based vaccines, with the aim of advancing this promising field.

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1. Introduction

Viral infections remain a significant global health threat that causes tissue damage, immune disorders, organ damage and even death [1,2]. Against viruses with high replication and mutation rates, vaccination remains the most effective strategy for preventing and combating viral infections. Traditional vaccines, which include inactivated or attenuated whole viruses (*e.g.*, poliovirus), elicit robust immune activation and durable protection but carry inherent safety risks [3]. Subunit vaccines, comprising proteins, peptides, DNA, RNA, and other pathogen-derived components, address these safety concerns by eliminating the risk of pathogenicity [4–6]. However, their simplified molecular structures undergo rapid systemic clearance and fail to efficiently activate antigen-presenting cells (APCs), resulting in suboptimal antibody titers and inadequate immune memory [7].

Adjuvants constitute a class of substances that non-specifically enhance host immune responses to antigens. Following Glenny's 1926 discovery that aluminum salts potentiated diphtheria toxoid immunogenicity compared to individual antigens, alum became the first approved human adjuvant. While alum predominantly induces Th2-biased humoral immunity, it exhibits limited capacity for cellular immunity [8]. In addition, recombinant proteins and peptides are difficult to effectively activate the natural immune system, resulting in insufficient adaptive response, which drives the development of a new generation of adjuvants, including MF59, AS03, and AS01. By mimicking pathogen-associated signals, these adjuvants can effectively engage the immune system, significantly enhancing both Th1-type cellular immunity and antibody responses [9–12]. More recently, RNA-based antigens have garnered significant attention due to their design flexibility and rapid development potential. However, this class of antigens faces a unique set of challenges: Rapid enzymatic degradation, high

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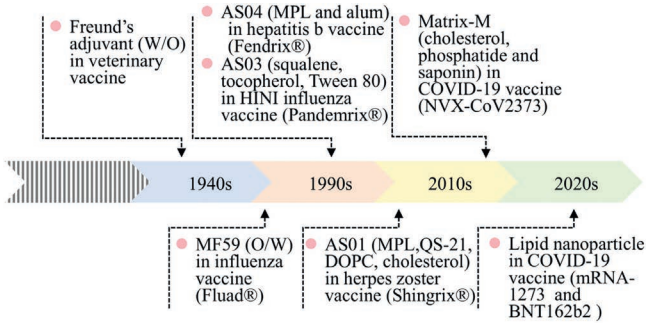


Fig. 1. The development of lipid-based adjuvants and delivery systems in antiviral vaccines.

systemic clearance, low bioavailability, and the critical requirement for efficient intracellular delivery [13]. These challenges have spurred the development of next-generation adjuvants centered on advanced delivery systems, notably lipid nanoparticles (LNPs) (Fig. 1). LNPs have become the pivotal delivery platform for mRNA vaccines [14]. Their core advantages lie in the ability to efficiently encapsulate and protect the fragile RNA antigen, facilitate uptake by immune cells, and optimize intracellular trafficking of the antigen. Furthermore, this delivery strategy substantially enhances antigen presentation efficiency and T/B cell activation, leading to a significant boost in overall immunogenicity [15–20].

With growing recognition of the essential role of adjuvants in modern vaccine formulations, a variety of adjuvant platforms have been developed to optimize antigen delivery and immunogenicity [21–25]. Compared with inorganic materials and viral vectors, lipid-based adjuvants are considered among the most flexible and promising platforms due to their excellent biocompatibility, high loading capacity, and ease of surface modification [26–31]. For example, AS01, used in the Shingrix herpes zoster vaccine, is a liposomal adjuvant that co-delivers monophosphoryl lipid A (MPL) and quillaja saponaria molina, fraction 21 (QS-21), facilitating colocalization and cellular uptake of both immunostimulants [32]. As an immunostimulatory lipid-based adjuvant, AS01 enhances innate immune activation and drives a potent Th1-biased response. Similarly, AS03, an oil-in-water emulsion used in pandemic influenza vaccines, relies on squalene-based lipid droplets to recruit innate immune cells and enhance local antigen presentation [33]. Lipid-based adjuvants not only function as immune enhancers but also serve as delivery platforms in vaccine systems [34]. LNPs self-assemble with negatively charged mRNA *via* electrostatic interactions, which is crucial for protecting the mRNA, as it is rapidly degraded by extracellular nucleases [35]. The most prominent examples are BNT162b2 and mRNA-1273, in which ionizable lipids not only protect the mRNA from enzymatic degradation but also facilitate endosomal escape through pH-responsive membrane destabilization, ensuring successful antigen expression in the cytosol [36]. Furthermore, lipid-based delivery systems are highly efficient in facilitating cellular uptake and intracellular trafficking, particularly when engineered with optimized surface charge and lipid composition [37].

In the face of the current severe viral epidemic landscape (*e.g.*, influenza, mpox virus), lipid-based adjuvants and delivery systems have become critical technological pillars for enhancing vaccine efficacy and curbing viral transmission. This review provides a comprehensive analysis of the application of lipid-based delivery systems in antiviral vaccines, encompassing liposomes, emulsions, LNPs, and expanded lipid adjuvants. Our coverage spans their design principles, formulation strategies, and immune activation mechanisms, with particular emphasis on clinically approved lipid carriers that enhance antigen stability, immune targeting, and synergistic adjuvant functions. We highlight emerging trends in lipid adjuvant development-such as novel lipid architectures, multifunctional systems, and modular platforms-positioned to address future viral threats.

2. Liposomes as adjuvants and delivery systems in antiviral vaccines

Liposome-based adjuvants are nanoscale vesicular systems composed mainly of phospholipids and cholesterol, widely used in antiviral vaccines to enhance antigen stability and immunogenicity (Table 1). By co-encapsulating immunostimulatory agents such as MPL and QS-21, they not only potentiate immune activation but also protect antigens from enzymatic degradation and prolong their systemic half-life. When properly engineered, liposomal adjuvants enable targeted delivery to lymph nodes and APCs, eliciting strong humoral and cellular immune responses. Owing to their excellent biocompatibility, structural versatility, and synergistic immunostimulation, representative systems such as AS01 and CAF01 (cationic adjuvant formulation 01) have shown outstanding efficacy in vaccines against herpes zoster, malaria, and human immunodeficiency virus (HIV).

2.1. AS01

Developed by GlaxoSmithKline (GSK) in the early 2000s, AS01 is a liposome-based adjuvant system containing MPL and QS-21. It forms hollow vesicles (~100 nm) composed of 1,2-dioleoyl-sn–glycero-3-phosphocholine (DOPC) and cholesterol (Fig. 2A). MPL activates Toll-like receptor 4 (TLR4), inducing proinflammatory signaling, while QS-21 triggers NLRP3 inflammasome activation and subsequent IL-1β/IL-18 release. Acting through these pathways, AS01 enhances both cellular and humoral immunity and enables tunable delivery *via* compositional adjustments. Following injection, AS01 and its associated antigen rapidly migrate to draining lymph nodes, where chemokine-mediated recruitment of dendritic cells (DCs) and activation of tissue macrophages amplify innate signaling and promote NK cell activation. This cascade efficiently primes antigen-specific T cells, drives B cell maturation, and establishes robust immune memory [38,39].

Hence, AS01 is suitable for vaccines that require strong cellular and/or humoral immunity, such as antiviral, antibacterial, and anti-tumor vaccines. AS01 is used in three approved vaccines: The malaria vaccine Mosquirix® (European Medicines Agency (EMA), 2015), the shingles vaccine Shingrix® (Food and Drug Administration (FDA), 2017), and the RSV vaccine AREXVY® (U.S. FDA, 2025). Shingrix®, administered intramuscularly in two doses, demonstrated 97.4% efficacy against shingles, increasing glycoprotein E (gE)-specific humoral and $CD4^+$ T cell responses by ~40-fold and 20-fold, respectively [41]. Compared to gE antigen alone, the AS01-adjuvanted formulation (gE/AS01) induced transient IL-6 and IL-1β production and markedly increased neutrophil, $Ly6C^{high}$ monocyte, and DC recruitment-by 53-, 230-, and 8.6-fold, respectively, within 24 h [42]. AS01 induces durable immune memory, maintaining high antibody seropositivity for 2–4 years post-immunization in vaccines such as RTS, S, gp120/NefTat, and HBs [43]. AS01 has demonstrated a favorable safety profile in clinical and post-marketing studies, with transient, controllable local reactions (pain, erythema, induration) as the main adverse effects.

2.2. Matrix-M

Matrix-M, developed by Novavax, is a nanoparticulate adjuvant composed of two saponin fractions co-formulated with cholesterol and phosphatidylcholine (Fig. 2B). These components self-assemble into cage-like nanoparticles (~40 nm) that retain potent

Table 1
The Information of on approved lipid-based adjuvants and delivery systems.

Lipid-based adjuvant and delivery systems	Name	Company	Composition	Mechanism	Applied vaccines and limitation
Liposomes	AS01	GSK	MPL, QS-21, DOPC, cholesterol	Draining lymph node aggregation; Th1-polarized immune response	Herpes zoster virus (Shingrix®), respiratory syncytial virus (AREXVY®); inflammatory side effects (pain, erythema, or induration)
	Matrix-M	Novavax	Quillaja saponaria, cholesterol, phosphatide	Th1-skewed adaptive response; activates NLRP3 inflammasome	COVID-19 (Novavax®); tenderness and pain at the injection site, fatigue, headache, and myalgia
Emulsions	MF59	Novartis	Squalene, Tween 80, Span 85	Inducing the release of danger-associated molecular patterns; facilitating germinal center formation	Seasonal influenza (Fluad®), A/H1N1 influenza (Focetria®, Celtura®); local injection-site reactions (pain, tenderness, and fatigue)
	AS03	GSK	Squalene, α-tocopherol, Tween 80	Activating NF-κB, enhancing cytokine secretion; activating B cells	A/H1N1 influenza (Pandemrix®), COVID-19 (Covifenz®); more obvious local inflammatory reaction (injection-site pain, redness, swelling, fever, and headache, narcolepsy)
	AF03	Sanofi	Squalene, Span 85, poly-oxyethylene cetylether	Enhance immune cell recruitment and antigen uptake	Previously used for A/H1N1 influenza (Humenza®); most are in the clinical research stage; the mechanism of action is not yet clear; limited safety data
	ISA 720/206	Seppic	Squalene, mannitol monooleate	Forming an antigen depot; Enhancing antigen presentation	Foot-and-mouth disease, avian influenza, *etc.* (Montanide® series); severe local adverse reactions; limited human use
LNPs	BNT162b2	BioNTech/Pfizer	DSPC, cholesterol, PEG2000-DMA, ALC-0159, ALC-0315	ALC-0315 and SM-102 encapsulates mRNA in LNPs and destroys the endosomal membrane, releasing mRNA in the cytoplasm through electrostatic interaction.	COVID-19; local inflammatory response
	mRNA-1273	Moderna	DSPC, Cholesterol, PEG2000-DMG, SM-102		

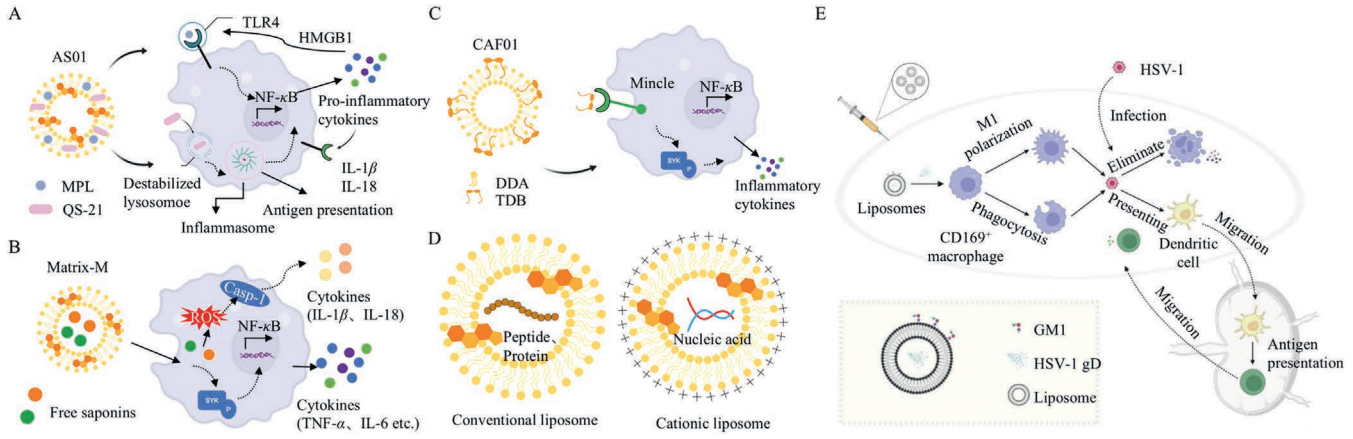


Fig. 2. The rational design of liposome adjuvant. (A) Schematic representation of the cellular mechanism signaling pathways triggered by AS01. (B) Schematic representation of the cellular mechanism signaling pathways triggered by Matrix-M. (C) Schematic representation of the cellular mechanism signaling pathways triggered by CAF01. (D) Schematic representation of conventional liposomes for peptide antigen delivery and cationic liposomes for nucleic acid delivery. (E) GM-gD-lip polarized CD169 macrophages toward M1 to eliminate the virus while cross-presenting antigens to $CD8^+$ T cells *via* DCs to activate adaptive immunity. Reproduced with permission [40]. Copyright 2023, Elsevier B.V.

immunostimulatory activity while reducing the intrinsic toxicity of free saponins through lipid stabilization [44]. Matrix-M rapidly activates innate immune cells at the injection site and in draining lymph nodes, promotes a Th1-biased adaptive response, enhances antigen presentation to $CD4^+$ and $CD8^+$ T cells, and activates the NLRP3 inflammasome, inducing IL-1β and IL-18 release [45].

Matrix-M was incorporated into the NVX-CoV2373 vaccine against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Clinical efficacy of this formulation was initially demonstrated in a phase 2 study conducted in South Africa and a phase 3 trial in the United Kingdom. These investigations confirmed that NVX-CoV2373 adjuvanted with Matrix-M was a potent addition to the existing portfolio of coronavirus disease 2019 (COVID-19) vaccines targeting the original strain, achieving an efficacy rate of 96.4% [46,47]. Matrix-M also enhanced the immunogenicity of a seasonal trivalent virosomal vaccine (TVV), doubling hemagglutination inhibition titers and improving protection relative to non-adjuvanted TVV. Moreover, in a UK phase 2a trial, the R21 malaria

vaccine adjuvanted with Matrix-M achieved 81.8 % sterile protection against *Plasmodium falciparum* infection [48].

Despite its favorable immunogenic profile, Matrix-M can elicit local reactogenicity, with the most commonly reported adverse events including tenderness and pain at the injection site, fatigue, headache, and myalgia.

2.3. CAF01

Developed by the Statens Serum Institut (SSI), CAF01 is a liposomal adjuvant composed of two key components: Dimethyldioctadecylammonium bromide (DDA) and trehalose-6,6-dibehenate (TDB) (Fig. 2C). These molecules self-assemble into uniform, nanoscale unilamellar vesicles with an average diameter of around 170 nm. Functioning as a depot at the site of administration, CAF01 extends antigen availability and induces strong Th1- and Th17-skewed immune responses, thus possessing long-term protection and immune memory functions [49–51]. It also functions as a potent immunostimulatory adjuvant. DDA, a cationic lipid, electrostatically binds negatively charged antigens, facilitating their delivery to APCs. TDB, a synthetic glycolipid that mimics mycobacterial cord factor derivatives, activates innate immunity *via* the Mincle receptor, initiating the FcRγ-Syk-CARD9 signaling cascade and triggering nuclear factor kappa-B (NF-κB) activation. At an optimal DDA:TDB ratio of 5:1, TDB intercalates into the DDA bilayer, enhancing hydrophilicity and imparting long-term physicochemical stability to the liposomes [52].

CAF01 currently has a limited range of applicable antigens, mainly focusing on specific recombinant protein vaccines, and remains in early clinical development. It has been primarily evaluated in vaccines for tuberculosis, HIV, and influenza prevention. The tuberculosis vaccine Ag85B-ESAT-6-CAF01 [53] is a two-vial formulation combining lyophilized recombinant antigen with CAF01 suspension. It induces multifunctional $CD4^+$ T helper cells with Th1 characteristics and elicits long-lasting humoral and cellular immunity. Okoth *et al*. [54] demonstrated that BALB/c mice receiving a subcutaneous CAF01-adjuvanted *P. yoelii* 17X vaccine achieved ~70 % survival against *P. yoelii* 17XL challenge, contrasting sharply with the <30 % protection rate in non-adjuvanted vaccine groups. Following heterologous rechallenge at 20 weeks post-immunization, these mice exhibited complete protection (100 % survival) with peak parasitemia levels below 0.1 %. This study establishes that CAF01-adjuvanted killed parasite vaccination provides significant primary infection protection and enhances long-term immunity against subsequent exposures, effectively modeling real-world protective responses.

In public data from early development stages and existing clinical studies, the safety of CAF01 is generally considered acceptable, with common adverse events being local injection site reactions or mild systemic reactions.

2.4. The current evolutionary direction of liposomes-based vaccines

Liposome adjuvants were initially used as immunopotentiators in vaccines, but this ignored the function of liposomes as drug delivery systems. The latest research has adjusted the formulation of liposomes to enable them to load antigens, realize precise targeted delivery and enhance immunity through reasonable surface modification.

2.4.1. Liposomes for delivering various types of antigens

The degree of antigen adsorption onto liposomes profoundly influences vaccine-induced immune responses. Studies show that non-adsorbed antigens co-administered with the adjuvant CAF01 elicit minimal T cell activation, whereas liposome-adsorbed antigens induce markedly stronger T cell responses, especially within the Th17 subset, highlighting liposomes as potent antigen carriers [55].

Conventional liposomes are nano- to microscale vesicles composed of phospholipid bilayers capable of encapsulating hydrophilic and lipophilic components, widely applied for peptide or protein antigen delivery in antiviral vaccines (Fig. 2D). For example, Teplensky *et al*. [56] designed a liposomal platform densely decorated with radially arranged CpG oligodeoxynucleotides (CpG ODN) for delivering recombinant SARS-CoV-2 receptor-binding domain (RBD) protein. Compared with conventional adjuvant formulations (Alum, AS01b, CpG ODN, AddaVax), this structured vaccine elicited a tenfold increase in functional antibody titers and achieved complete protection (100 % survival) in mice. Similarly, Shao *et al*. [57] engineered a cobalt porphyrin–phospholipid (CoPoP) and MPL-containing liposomal vaccine delivering the HIV membrane proximal external region (MPER) peptide, inducing ~100-fold higher anti-MPER IgG titers than conventional Alum or Montanide formulations.

Cationic liposomes composed of positively charged lipids, such as 1,2-dioleoyloxy-3-trimethylammoniumpropane (DOTAP) [57] enhance cellular uptake *via* electrostatic interactions with negatively charged membranes and stabilize anionic nucleic acids, making them effective nucleic acid vaccine carriers [58]. Utilizing DOTAP-based liposomes, Peletta *et al*. [59] incorporated a plasmid DNA (pCMVkan-S) encoding the spike (S) protein, significantly enhancing neutralizing antibody and T cell responses compared to naked DNA. Melo *et al*. [60] employed DOTAP liposomes to deliver eOD-GT8 60-mer–encoding replicons, an HIV immunogen, achieving elevated antigen-specific antibody titers and increased germinal center B cells compared with protein vaccination.

2.4.2. APCs-targeted liposomal adjuvant delivery systems

APCs, including DCs and macrophages, initiate T and B cell activation. Targeting APCs enhances antigen recognition and processing, prolongs immune responses, and improves vaccine precision and safety.

Macrophages act as frontline defenders during viral infection, with $CD169^+$ macrophages playing key roles in antigen presentation. Shen *et al*. [40] designed GM-gD-lip, a GM1-based liposomal vaccine encapsulating HSV-1 glycoprotein D, to target $CD169^+$ macrophages. Subconjunctival administration reduced HSV-1 titers and herpes simplex keratitis (HSK) symptoms (Fig. 2E), promoted M1 macrophage polarization, and enhanced DC-mediated cross-presentation to $CD8^+$ T cells, thereby strengthening adaptive immunity.

Since antigen processing occurs mainly in the endoplasmic reticulum (ER) of APCs [61], vaccines targeting both lymph nodes and the ER can improve cross-presentation *via* the ER-associated degradation (ERAD) pathway. You *et al*. [62] developed a mannosylated liposomal system (PM-LIPO) incorporating DSPE-PEG-Man and DSPE-PEG-Par to achieve receptor-mediated APC targeting and ER-directed antigen delivery. When loaded with SARS-CoV-2 S protein and CpG adjuvant, PM-LIPO induced >40 % viral inhibition and sustained neutralizing antibody titers up to eight weeks post-immunization in mice.

Such strategies enhance the specificity and efficiency of antigen presentation, thereby amplifying antigen-specific immune responses. Targeted liposomal adjuvants thus hold strong translational potential as next-generation vaccine platforms, improving antigen stability, delivery precision, and immunogenicity.

3. Emulsions as adjuvants and delivery systems in antiviral vaccines

Emulsion adjuvants enhance vaccine immunogenicity through an oil-water emulsification system composed of an oil phase (*e.g.*,

squalene, mineral oil), an aqueous phase (*e.g.*, PBS, citrate), and an emulsifier (*e.g.*, Tween 80, Span 85) [63]. Some formulations also include immunostimulatory enhancers such as α-tocopherol. Based on emulsification structure, they are classified as water-in-oil (W/O), oil-in-water (O/W), or water-in-oil-in-water (W/O/W) types [64]. W/O emulsions, such as Freund's adjuvant, exhibit prolonged oil-phase retention, often causing local adverse effects including ulcers, granulomas, and allergies [65], and are therefore limited to veterinary use. In contrast, O/W emulsions using biodegradable and biocompatible oils offer superior safety and tolerability [66], supporting their broader application. A notable advantage of O/W systems is the ability to store antigen and adjuvant separately and mix them immediately before administration [65]. Emulsion adjuvants enhance immune responses *via* multiple mechanisms, including antigen depot formation for sustained release, induction of pro-inflammatory signaling to activate innate immunity, and promotion of antigen presentation, thereby amplifying both humoral and cellular immunity. They also enable antigen dose sparing and significantly increase antigen-specific antibody titers, outperforming alum adjuvants in several respects [67]. Several emulsion adjuvants have been approved for clinical use (Table 1) [68] and are expected to become a mainstream platform in future vaccine development.

3.1. MF59

MF59, developed by Novartis, is a squalene-based O/W emulsion originally designed as a delivery vehicle for the immunostimulant MTP-PE [69]. Subsequent studies revealed its strong immunostimulatory properties, leading to its use as a standalone adjuvant. MF59 consists of squalene, nonionic surfactants (Tween 80, Span 85), and citrate buffer, forming negatively charged particles (~160 nm) [65]. Unlike alum or Freund's adjuvant, MF59 does not form a persistent depot but instead induces a transient pro-inflammatory milieu rich in DAMPs (ATP, dsDNA, uric acid), cytokines, and chemokines, which recruit neutrophils, monocytes, and DCs to amplify antigen presentation (Fig. 3A) [70]. Recruited immune cells carrying antigen/adjuvant complexes migrate to draining lymph nodes, where MF59 promotes monocyte-to-DC differentiation, germinal center formation, and antibody affinity maturation. MF59 enhances humoral immunity *via* MyD88/ASC-dependent pathways [66] and $CD8^+$ T cell activation through RIPK3, and can induce antigen-specific immunity independently of $CD4^+$ T cell help [68]. Importantly, MF59's immunogenic effects rely on the intact emulsion formulation, as its individual components lack comparable adjuvanticity [71].

MF59 has demonstrated robust efficacy as an adjuvant in various licensed vaccines, including influenza and recombinant protein-based COVID-19 vaccines [68]. Fluad®, approved in Italy in 1997 for individuals ≥65 years, reduces influenza-related hospitalizations by up to 58.5% [76] and its pediatric formulation, Fluad Pediatric®, was later approved for children aged 6 months-2 years [77]. MF59 can be applied to a variety of antigens with different physicochemical properties, ranging from monomeric (HIV gp120) to particulate (HBV surface antigen) in nature, and from soluble gD2 antigen of HSV to insoluble hemagglutinin (HA) antigen of influenza virus in water [78]. In addition, MF59 not only reduces the amount of antigen in vaccines, but also exhibits a favorable safety profile. Compared with non-adjuvanted vaccines, MF59-adjuvanted formulations are indeed associated with local injection-site reactions such as pain, tenderness, and fatigue; however, serious side effects, including narcolepsy, that are closely associated with MF59 adjuvant have not been reported [68,79,80]. Despite clinical success, its precise mechanisms remain incompletely defined, as most insights derive from murine studies. Further research is needed to clarify how administration routes and demographics affect MF59-mediated immunity [68].

3.2. AS03

AS03, developed by GSK in 2009, is a squalene-based O/W emulsion adjuvant comprising squalene, α-tocopherol, and Tween 80 [81], with a mean particle diameter of ~160 nm (Fig. 3A). AS03 enhances immune activation through multiple mechanisms: It triggers NF-κB signaling at the injection site, inducing cytokine and chemokine release, which in turn facilitates the recruitment of monocytes, macrophages, and DCs to draining lymph nodes. This promotes antigen uptake by APCs and stimulates B cell activation *via* $CD4^+$ T cell help, ultimately enhancing antigen-specific antibody production [65]. Similar to MF59, AS03 is formulated with squalene as a core component [82] and predominantly elicits a Th2-skewed immune response, with relatively limited induction of Th1-type immunity [83]. What distinguishes AS03 is the addition of α-tocopherol, which is the most biologically active core ingredient in vitamin E [84]. Studies indicate that compared to AS03 with α-tocopherol removed, the α-tocopherol-containing version can further increase cytokine levels, shorten the migration time of early eosinophils and neutrophils to draining lymph nodes, enhance antigen load in monocytes, and increase antibody levels [82].

AS03 is primarily utilized with glycoprotein antigens located on the surface of enveloped viruses, such as influenza virus HA and the SARS-CoV-2 spike protein. Its advantage is that it enhances the immunogenicity of antigens and promotes immune responses in people with weaker immunoreactivity. In addition, the addition of AS03 adjuvant can reduce the amount of vaccine antigens used, thereby reducing production costs [85]. Consequently, AS03 is now widely used in various antiviral vaccines, such as Pandemrix® for influenza A (H1N1) and avian influenza vaccines for H5N1 [83,86]. Compared to non-adjuvanted vaccines, these vaccines induce a stronger humoral immune response. A randomized controlled trial showed that 28 days after a single primary dose of a chimeric HA influenza vaccine, the Geometric Mean Concentration (GMC) of anti-H1 stem antibodies induced by the AS03-adjuvanted group was ~5.3 times that of the non-adjuvanted group under the same conditions [87]. Furthermore, Medicago Inc. of Canada, in collaboration with GSK, developed a COVID-19 vaccine, Covifenz®, which combines AS03 with plant-derived coronavirus-like particles (CoVLP). This combination induces potent and durable neutralizing antibody levels. After two doses, the geometric mean titer (GMT) of neutralizing antibodies induced in the AS03-adjuvanted group was over 100 times higher than that of the non-adjuvanted group [88], and it showed a balanced T cell response [89]. AS03 adjuvant has been widely used in various vaccines. However, beyond the common adverse events attributable to its inflammation-inducing effects, such as injection-site pain, redness, swelling, fever, and headache [65], it is of particular concern that the AS03-adjuvanted H1N1 vaccine Pandemrix® was associated with an increased incidence of narcolepsy among adolescents in Sweden and Finland within 3–6 months following vaccination during the 2009 pandemic [90,91]. Although the exact mechanism remains unclear, several studies have explored the potential link between Pandemrix® and narcolepsy. One study found that the onset of narcolepsy after Pandemrix® vaccination may be related to an autoimmune response triggered by the combination of AS03 and the antigen [67,77,91].

3.3. ISA 720/206

Freund's adjuvant is a W/O emulsion composed of paraffin oil and lanolin, and exists in two forms: Complete Freund's adju-

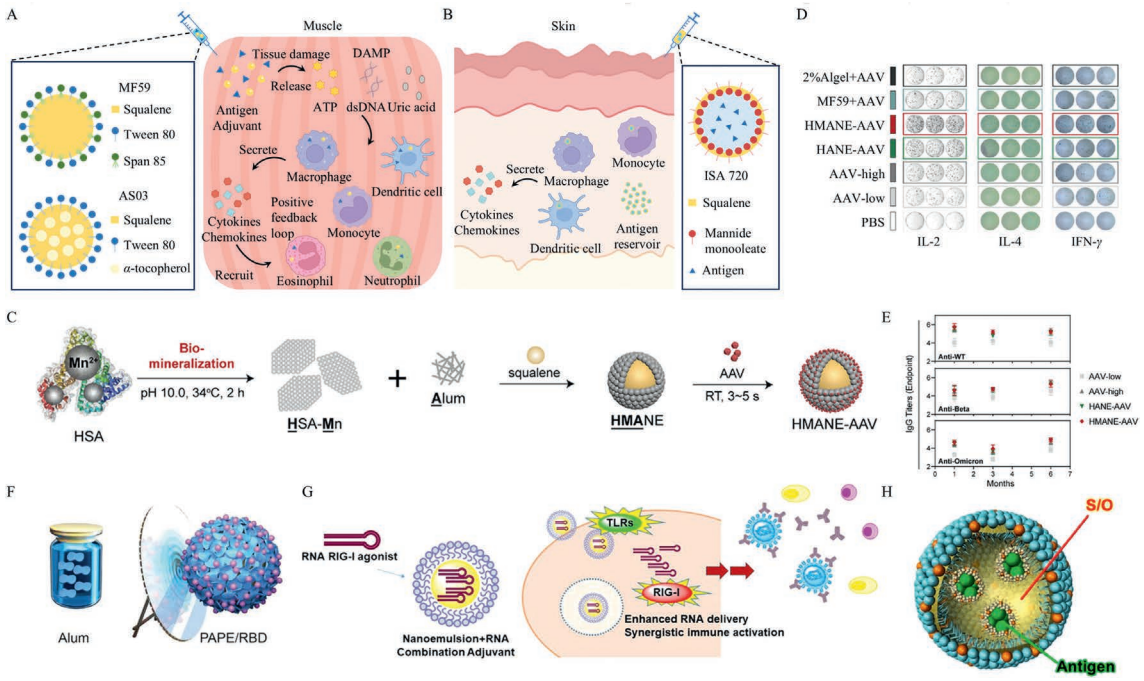


Fig. 3. The rational design of emulsion adjuvant. (A) Schematic diagram of the structure and mechanism of MF59 and AS03. (B) Schematic diagram of the structure and mechanism of ISA 720. (C) Schematic diagram of HMANE-AAV preparation. (D) The representative ELISPOT images of IL-2, IL-4, and IFN-γ spot-forming dots. (E) Serum IgG antibody response to variable strains over time. Copied with permission [72]. Copyright 2025, Wiley-VCH. (F) Schematic illustration of PAPE strategy. Copied with permission [73]. Copyright 2020, Wiley-VCH. (G) Schematic diagram of emulsion and RIG-I activating RNA as combined adjuvants for influenza virus. Copied with permission [74]. Copyright 2021, American Chemical Society. (H) Schematic representation of the S/O/W emulsion. Copied with permission [75]. Copyright 2022, Elsevier Ltd.

vant (CFA), which includes heat-killed mycobacterium tuberculosis, and incomplete Freund's adjuvant (IFA), which lacks the mycobacterial component [92]. To mitigate granuloma formation and chronic inflammation, Seppic developed Montanide™ ISA 720 by replacing mineral oil with metabolizable squalene and using mannide monooleate as an emulsifier [93]. ISA 720 encapsulates water-soluble antigens within the oil phase, forming a depot that allows sustained antigen release and prolonged immune stimulation (Fig. 3B) [94]. It is compatible with diverse purified or synthetic antigens and induces strong antibody and CTL responses [95]. For instance, CpG + ISA 720 induced a Th1-skewed anti-HCV response with enhanced IFN-γ secretion and IgG2a-dominant immunity [96]. Currently, ISA 720 is primarily used in veterinary vaccine adjuvants, such as antiviral vaccines for poultry, fish, and other livestock. Additionally, this emulsion adjuvant is being investigated for use against HIV, malaria, and tumors [97]. However, its prolonged retention at the injection site often causes significant local adverse reactions, including pain, swelling, and sterile abscesses, limiting its application in human vaccines.

W/O/W emulsions have emerged as improved alternatives with greater injectability and reduced local side effects. Montanide™ ISA 206 VG, widely used in veterinary vaccines, elicited higher and longer-lasting neutralizing antibody titers against foot-and-mouth disease virus than alum-based formulations [98].

3.4. The current evolutionary direction of emulsions-based vaccines

While conventional emulsion adjuvants have proven successful, current research is focused on developing next-generation platforms to overcome their inherent limitations and further enhance vaccine efficacy. These development trends primarily aim to improve antigen stability, broaden the types of immune responses induced, and increase overall potency. Key strategies include innovating the physical structure of the emulsion for superior antigen protection, developing novel stabilization platforms that offer multifunctionality, and employing synergistic combinations of adjuvants to engage multiple immune pathways for more comprehensive protection.

3.4.1. Pickering emulsions

Pickering emulsions, stabilized by solid particles at the oil-water interface, prevent droplet coalescence, improve antigen protection, and promote antigen-APC interactions [99]. They mimic pathogen-like dynamics, enhancing antigen uptake and cross-presentation. In recent years, pickering emulsions have become a new platform for vaccine adjuvants and carriers due to their unique interfacial stabilization mechanism and strong immunostimulatory properties, especially in the development of antiviral vaccines, and have broad prospects for clinical translation.

As the functional characteristics of Pickering emulsions are largely governed by the physicochemical properties of the solid particles used, tailoring the particulate stabilizers allows for tunable immunological outcomes. Guo *et al.* [72] engineered a metal-based Pickering emulsion, termed HMANE, for adeno-associated virus (AAV) delivery (Fig. 3C). This system employed HAS-Mn nanoparticles and alum to stabilize a squalene core, enabling efficient AAV adsorption onto the emulsion interface. HMANE markedly improved AAV biodistribution, retention, and internalization. Moreover, it activated the cyclic GMP-AMP synthase-stimulator of interferon genes (cGAS-STING) pathway, upregulated AAV transgene expression, and promoted strong cellular immune responses, as evidenced by elevated cytokine levels (IL-2, IL-4, IFN-γ) (Fig. 3D). Upon immunization against various SARS-CoV-2 variants, HMANE-AAV induced high-titer, cross-reactive antibodies, demonstrating broad protective efficacy (Fig. 3E). Similarly, Peng *et al.* [73] developed an alum-stabilized squalene Pickering emulsion (PAPE) that enhanced DC uptake and cross-presentation (Fig. 3F). Pan *et al.* [100] constructed a mannose-functionalized chitosan nanoparticle (MCNP)-stabilized emulsion achieving 54.1 % cellular uptake and potent humoral and cellular responses with strong

antigen retention, highlighting its promise as a subunit vaccine platform.

3.4.2. Combined adjuvant emulsions

Traditional emulsions (*e.g.*, MF59, AS03) enhance humoral immunity but have limited ability to induce cellular responses. Incorporating innate immune agonists can address this limitation. One study showed that although the O/W emulsion adjuvant IDRI SE alone could enhance antibody responses, its ability to activate $CD8^+$ T cells was limited; however, the addition of the TLR9 agonist CpG activated inflammatory pathways, markedly enhanced CTL activity, promoted the secretion of pro-inflammatory cytokines (IFN-γ and IL-2), and reduced the proportion of regulatory T cells (Tregs) by ~20% [101]. In addition, natural infection with the influenza virus activates multiple pattern recognition receptors (PRRs). Therefore, combining multiple adjuvants can more accurately mimic natural infection and is expected to enhance immunogenicity and broaden the protective range against influenza viruses through multiple mechanisms [64,102]. Baker's group [74] combined two mechanistically complementary adjuvants for an intranasal influenza vaccine (Fig. 3G). The oil-in-water nanoemulsion (NE), primarily composed of cetylpyridinium chloride, Tween 80, ethanol, soybean oil, and water, can activate TLRs, stimulate immunogenic cell death, and enhance cellular uptake of the antigen, thereby achieving a balanced Th1/Th2/Th17 response upon intranasal administration. In contrast, the RNA-type RIG-I agonist strongly induces IFN-I and a Th1-polarizing response. The combined NE/RNA adjuvant can synergistically increase IFN-β secretion, promote antibody affinity maturation, and enhance Th1 and Th17 cellular immunity.

3.4.3. S/O/W emulsions

In conventional O/W emulsions, antigens are often unstable or prematurely released. To overcome this limitation, Yoshiro *et al.* [75] designed a solid-in-oil-in-water (S/O/W) emulsion by encapsulating the antigen as solid nanoparticles within the squalene oil phase, significantly improving antigen encapsulation efficiency and stability (Fig. 3H). Experimental data showed that even after one week of incubation in different media and buffers, the encapsulation rate of antigen remained above 60%. Through this design, the antigen can be more effectively co-delivered with the oil droplets to APCs, thereby inducing a stronger immune response. *In vivo* experiments further confirmed its superiority, as the S/O/W emulsion induced significantly higher serum IgG levels than the O/W emulsion and effectively activated CTLs to promote cellular immunity. Compared with the antigens in the aqueous phase of O/W emulsions that are rapidly transported to lysosomes and degraded, the key advantage of S/O/W emulsions as vaccine carriers is that the antigens are encapsulated in the oil phase and the antigens and oil-based adjuvants are delivered to APCs at the same time, which increases the number of APCs that capture antigens at the injection site and reduces the co-localization of antigens and lysosomes, thereby effectively activating humoral immunity and cellular immunity. As a novel emulsion adjuvant, the S/O/W emulsion has good biosafety and provides an innovative delivery platform for the development of antiviral vaccines.

4. LNPs as delivery systems in antiviral vaccines

Lipid nanoparticles (LNPs) have become the leading platform for nucleic acid delivery, offering significant potential for next-generation antiviral vaccines. Unlike liposomes or micelles, LNPs are specialized lipid-based nanocarriers that protect nucleic acids from degradation, promote cellular uptake and intracellular trafficking, and enable efficient endosomal escape for cytosolic delivery. LNPs are typically ~100 nm-sized carriers composed of four components: An ionizable cationic lipid, a helper phospholipid, cholesterol, and a PEGylated lipid (Fig. 4A). The ionizable lipid plays a pivotal role-its amine groups protonate under acidic conditions, allowing electrostatic complexation with nucleic acids and subsequent ion-pair formation with endosomal lipids. This process destabilizes the endosomal membrane, facilitating fusion and cargo release into the cytosol. Owing to their high transfection efficiency, scalable production, nanoscale uniformity, and serum stability, LNPs have become a cornerstone platform for antiviral vaccine delivery.

4.1. BNT162b2

The BNT162b2 vaccine, developed by BioNTech in partnership with Pfizer, was the first to receive Emergency Use Authorization (EUA) from the FDA on December 11, 2020 [104]. This vaccine consists of a nucleoside-modified mRNA encoding the full-length, prefusion-stabilized SARS-CoV-2 spike protein, encapsulated within LNPs. The LNP formulation includes ALC-0315, DSPC, cholesterol, and ALC-0159 at a molar ratio of 46.3:9.4:42.7:1.6, respectively (Fig. 4B) [105]. ALC-0315, a biodegradable ionizable lipid featuring ester linkages and protonatable amines, enables effective mRNA encapsulation (pK_a, 6.09) [106], and facilitates endosomal escape under acidic conditions through electrostatic disruption of the endosomal membrane. Following administration, muscle cells take up and translate the mRNA, presenting the spike protein to APCs. This leads to the activation of naïve T cells, their differentiation into effector and CTLs, and concurrent B cell maturation driven by T follicular helper cells, culminating in robust antibody production. Clinical trials demonstrated that BNT162b2 conferred 52% protection 12 days post-initial dose and 95% efficacy following the second dose administered 3–4 weeks later in SARS-CoV-2-naive individuals [107].

4.2. mRNA-1273

The Moderna vaccine (mRNA-1273), granted EUA by the World Health Organization (WHO) on April 30, 2021, employs a comparable LNP platform comprising SM-102, DSPC, cholesterol, and PEG2000-DMG at a molar ratio of 50:10:38.5:1.5 (Fig. 4B) [108]. SM-102, also a biodegradable ionizable lipid, promotes nucleic acid encapsulation and endosomal escape, with a measured pK_a of 6.68 [106]. The mRNA encodes the SARS-CoV-2 spike glycoprotein, which is central to viral entry and infection [109]. Following administration, muscle cells take up and translate the mRNA, presenting the spike protein to APCs. This leads to the activation of naïve T cells, their differentiation into effector and CTLs, and concurrent B cell maturation driven by T follicular helper cells, culminating in robust antibody production [110]. Two-dose administration of mRNA-1273 demonstrated >90% efficacy in preventing symptomatic COVID-19. Observational data further indicate that both mRNA vaccines provide strong protection against the original strain and early variants (alpha, beta), though efficacy is reduced against the delta variant [111].

BNT162b2 and mRNA-1273 have shown acceptable safety in the application of COVID-19 vaccines, but there are still potential side effects, such as local inflammatory response. This is attributed to the ability of ionizable cationic lipids to perturb cellular and nuclear membranes, causing cellular damage. To address this substantial shortcoming of ionizable cationic lipids, for one thing, biodegradable alternatives may be introduced that are rapidly degraded *in vivo* to preferably endogenous metabolites; for another, optimizing the organ or cell targeting of ionizable cationic lipids may reduce non-specific uptake, thereby reducing local toxicity.

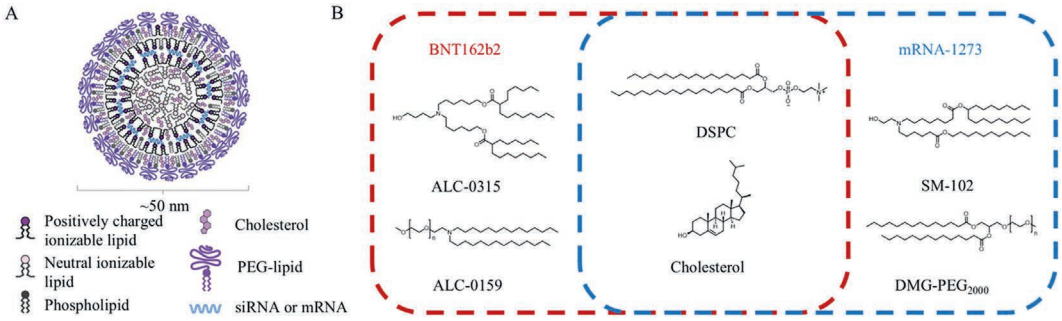


Fig. 4. The structure and composition of LNPs. (A) Illustration of the components of LNPs. Copied with permission [103]. Copyright 2018, American Chemical Society. (B) Structures and compositions of lipids applied to BNT162b2 and mRNA-1273.

4.3. The current evolutionary direction of LNPs-based vaccines

Despite the unique advantages of higher delivery efficiency and easier scale-up production in comparison with other lipid-based nanocarriers, LNPs loaded with mRNA still face several critical challenges. First, the LNP compositions indicate a critical challenge for the cytosolic delivery of mRNA to targeted cells in the clinic. mRNA-1273 and BNT162b2 fail to target APCs directly and the interaction between mRNA and non-APCs at the intramuscular injection site may consequently elicit undesirable side effects. Second, sufficient mRNA translation within APCs is crucial for robust immune activation, requiring LNPs to optimize both cellular uptake and endosomal escape. However, LNP-mediated nucleic acid delivery efficiency remains below 2 % [112,113] and needs further improvement. Recent studies have demonstrated that it is possible to enhance the targeting capabilities and transfection efficiency by refining the composition of LNP.

4.3.1. Design and screening of novel ionizable cationic lipids for efficient delivery of nucleic acid

The rational design and screening of ionizable cationic lipids have attracted growing interest due to their critical roles in nucleic acid encapsulation within LNPs and endosomal escape for cytosolic delivery. Vitamin B5 contains three modifiable groups-primary hydroxyl (RA), secondary hydroxyl (RB), and carboxylic acid (RC)-providing versatile scaffolds for lipid design. Yoo *et al.* [114] leveraged vitamin B5 as the core to synthesize ionizable lipids classified into three structural groups (I7X, I8X, I9X) based on tail–headgroup conjugation: (1) I7X, with RA as the headgroup and RC-linked tail; (2) I8X, with RA as headgroup and tails at RB and RC; and (3) I9X, with RC as headgroup and tails at RA and RB (Fig. 5A). The optimal formulation, LNP 5097, was identified through evaluation of size, surface pK_a, transfection efficiency, and *in vivo* immunogenicity. LNP 5097, composed of the ionizable lipid I97, 6,6′-trehalose dioleate (TDO), DMG-PEG2000, 1,2-dioleoyl-*sn*–glycero-3-phosphoethanolamine (DOPE), and *n*–butyl lithocholic acid (L-Bu) at a molar ratio of 25:25:1.5:10:38.5, exhibited a diameter of 92 ± 0.7 nm, high monodispersity, and a pK_a of 6.23. Compared with SM-102-based LNPs, LNP 5097 achieved 1.37-fold higher mRNA transfection and induced stronger HA mRNA vaccine–elicited neutralizing antibody responses in mice. Given the spleen's central role in adaptive immunity, Dong *et al.* [115] developed a class of imidazole-based ionizable lipids (IMILs) and performed structural optimization *via* a high-throughput screen of a multidimensional IMIL library containing diverse headgroups, tail architectures, and linker chemistries. Through *in vivo* screening, A3B7C2 emerged as a lead candidate, achieving spleen-targeted mRNA delivery with an exceptional 98 % transfection efficiency in splenic tissue. Furthermore, A3B7C2-formulated LNPs displayed potent transfection capabilities in splenic DCs. Comparative analyses revealed that A3B7C2-based LNPs achieved 2.8- and 12.9-fold higher mRNA expression in the spleen compared to SM-102 and DLin-MC3-DMA-based systems, respectively.

4.3.2. Ionizable cationic lipids with immunological adjuvant potential

Beyond serving as delivery vehicles, LNPs can recruit immune cells at the injection site, trigger local inflammation, and prime early immune responses [118], highlighting their inherent adjuvant-like properties. Zhang *et al.* [116] synthesized ionizable aminolipids (SALs) with intrinsic STING activation capacity. Among them, SAL12 induced strong IFN-β production in BMDCs even without mRNA. Immunization with SARS-CoV-2 spike mRNA-loaded SAL12-LNPs (SAL12 ΔS-LNPs) increased S1-specific IgG titers 5- and 3-fold compared to ALC-0315 LNPs at 4 and 10 weeks, respectively, confirming SAL12's potent adjuvanticity (Fig. 5B). Similarly, Miao *et al.* [119] synthesized and evaluated the immunostimulatory effect of over 1000 lipid formulations by using a one-step three-component reaction. The top candidates shared key structural traits: Unsaturated tails, dihydroimidazole linkers, and cyclic amine headgroups. Particularly, six-membered piperidinyl rings activated STING independently of MYD88 (TLR7/8) or RLR (RIG-I, MDA5) signaling. These STING-responsive lipids enhanced intracellular activation through improved endocytic uptake and cytosolic trafficking.

NLRP3, a key inflammasome sensor, activates caspase-1 and drives IL-1β and IL-18 secretion [120]. Zhou *et al.* [121] introduced a nanoparticle-based lipid adjuvant (NLA) platform derived from a structurally diverse library of 124 ionizable lipids (ILs). *In vitro* assays identified R2C18–2 NLA as a lead candidate capable of activating NLRP3, as evidenced by its induction of IL-1β production in BMDCs. *In vivo*, co-administration of R2C18–2 NLA with the RSV pre-fusion (pre-F) antigen *via* intramuscular injection elicited strong cellular and humoral immune responses, notably expanding both central and effector memory T cell subsets. Challenge experiments demonstrated that this formulation conferred protection comparable to AS01e-like liposomal adjuvants.

Toll-like receptor (TLR) agonists act as strong immune adjuvants by promoting DC maturation, enhancing antigen presentation, and stimulating pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), thereby bridging innate and adaptive immunity [122]. Han *et al.* [123] synthesized an ionizable lipid adjuvant, C12-TLRa, *via* ring-opening of C12 epoxide with an amine-functionalized TLR7/8 agonist. C12-TLRa imparted dual functionality-enhanced mRNA delivery and TLR7/8 agonism. SARS-CoV-2 mRNA vaccines formulated with SM-102/TLRa LNPs induced 5.4-fold higher anti-RBD IgG titers and robust Th1-biased immune responses compared to standard SM-102 LNPs.

4.3.3. Construction of selective organ-targeting LNPs

Unmodified LNPs exhibit strong liver tropism following intravenous administration, limiting their application to hepatic dis-

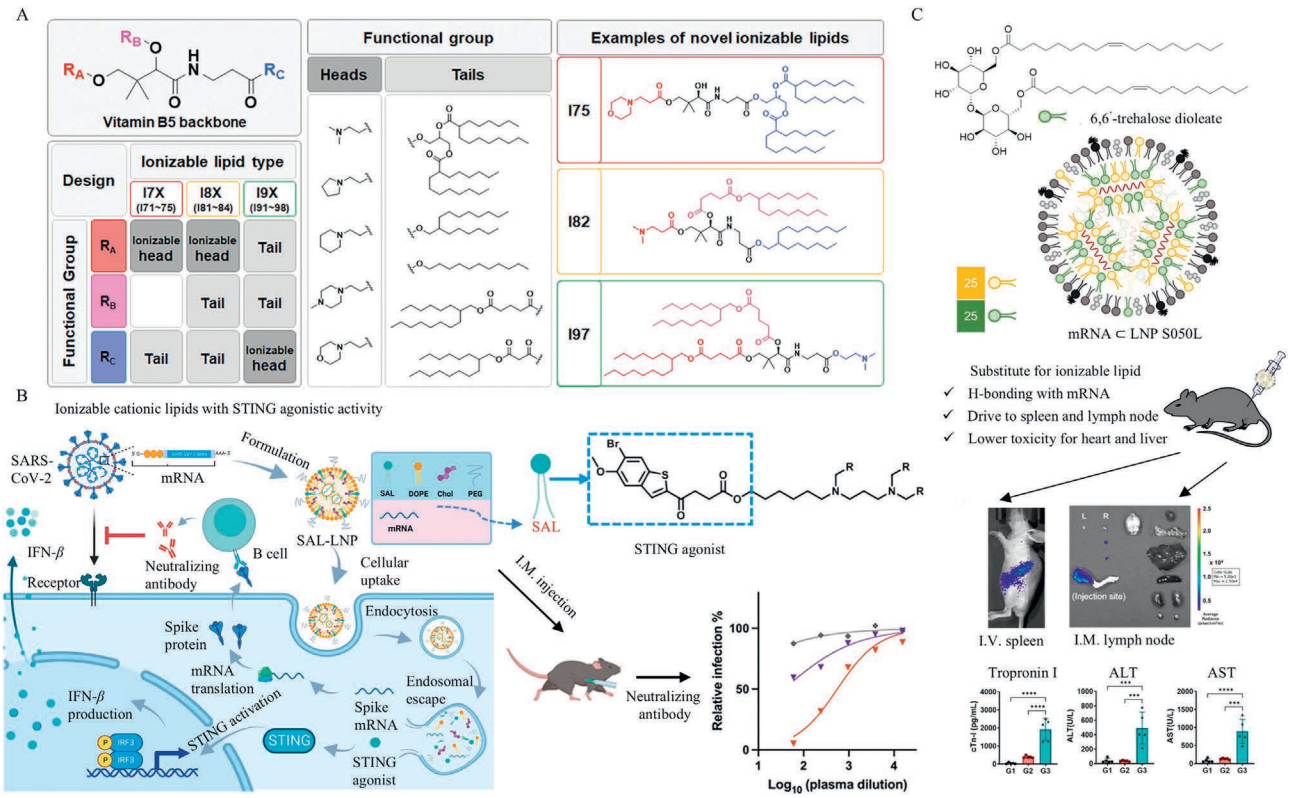


Fig. 5. The rational design of LNPs and application for the treatment of infectious diseases. (A) Structural design and representative architectures of vitamin B5-derived ionizable lipids. The three ionizable lipid series were designed according to the direction in which the ionizable heads and tails were functionalized at each functional group site: I7X (ionizable head at RA and tail at RC), I8X (ionizable head at RA and two tails at RB and RC), and I9X (ionizable head at RC and two tails at RA and RB). Copied with permission [114]. Copyright 2020, Wiley-VCH. (B) Schematic illustration of SAL-LNPs induced STING activation, mRNA delivery and humoral immune response. Reproduced with permission [116]. Copyright 2023, American Chemical Society. (C) Schematic illustration of novel LNP S050L based on trehalose glycolipids. A partial substitution of ionizable lipids with trehalose glycolipids maintained high efficacy in mRNA expression and immune response. The toxicity to the heart and liver was reduced compared to Con-LNPs and the mRNAs encapsulated within LNP S050L were traced in the lymph node and spleen. Reproduced with permission [117]. Copyright 2024, Elsevier B.V.

eases and vaccines [124]. For mRNA tumor vaccines, efficient antigen expression by professional APCs is essential, as translation by non-APCs may cause cytotoxicity [125]. To fully leverage their potential in antiviral vaccines, it is essential to develop strategies to enhance delivery to targeted organs or tissues beyond the liver for maximum efficacy and minimum toxicity.

Rationally engineered mRNA-LNPs targeting APCs show great promise for infectious disease vaccines. Bae *et al.* [117] developed trehalose glycolipid-based LNPs (S050L) that induced HA mRNA-elicited immune responses comparable to SM-102 LNPs but with improved lymphatic trafficking, accumulation in lymph nodes and spleen, and reduced systemic toxicity (Fig. 5C). Similarly, Lei *et al.* [126] synthesized a mannose-modified ionizable lipid (Man-lipid) incorporated as the fifth LNP component to form "sweet" LNPs (STLNPs). STLNPs-Man achieved fourfold higher DC uptake than ALC-0315 LNPs *via* mannose-CD206 interactions, markedly enhancing mRNA delivery to DCs. To further improve targeting, Cao *et al.* [127] developed DC membrane-coated nanoparticles (DCMNPs) composed of mRNA, ionizable lipid YK009, and DOPE. The positive charge of YK009 facilitated mRNA encapsulation and cytosolic release, while the DC membrane shell conferred lymphoid homing and enhanced biodistribution. A three-dose DCMNP-based SARS-CoV-2 mRNA vaccine elicited strong Spike-specific IgG, high neutralizing titers, and Th1-skewed cellular immunity.

In summary, LNPs significantly align with clinical requirements for therapeutic nucleic acids in the treatment of infectious diseases. Ongoing advancements aim to enhance their transfection efficiency and achieve precise targeting of APCs within lymph nodes or spleen to reduce adverse effects and optimize therapeutic outcomes.

5. Other lipid-based adjuvants and delivery systems in antiviral vaccines

5.1. Peptide-lipid conjugate based antiviral vaccines

Peptide-based vaccines offer distinct advantages over conventional platforms but often suffer from low immunogenicity, necessitating adjuvant use. To address this, lipopeptides have emerged as self-adjuvanting systems composed of a hydrophobic lipid chain covalently linked to a hydrophilic peptide head group. This amphiphilic architecture promotes supramolecular self-assembly in aqueous media, enhancing antigen presentation and immune activation [128].

Certain lipid moieties, such as tripalmitoylated cysteine (Pam3C) and dipalmitoylated cysteine (Pam2C), act as TLR2 agonists that trigger immune signaling upon antigen conjugation. Zeng *et al.* developed influenza vaccine candidates by linking Pam2C to the matrix protein 2 ectodomain (M2e) of influenza A virus. The resulting M2e-Pam2C lipopeptides elicited strong antigen-specific antibody responses in mice without additional adjuvants [129]. Lipopeptides can also serve as adjuvants to enhance the immunogenicity of co-administered antigens. Bao *et al.* synthesized amphiphilic lipopeptide adjuvants (LAAs) for co-delivery with monkeypox virus (MPXV) antigens. Among them, C16-GCV2E3 (C16), characterized by a twisted band-like nanostructure, markedly en-

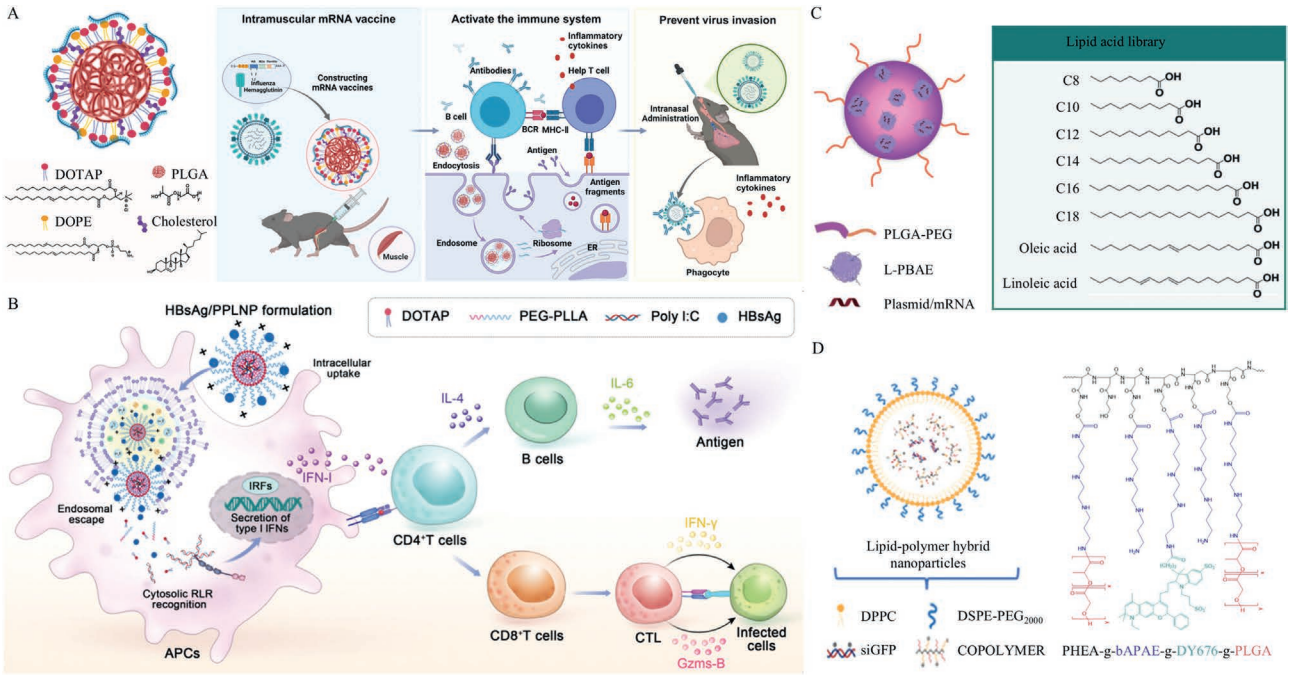


Fig. 6. The composition of the lipid-polymer hybrid based antiviral vaccines and their mechanism for enhancing cellular immunity. (A) The structure of lipid-polymer hybrid nanoparticles (PDCD) nanoparticles and its immune responses. Reproduced with permission [133]. Copyright 2024, American Chemical Society. (B) Constituents and immunoenhancing mode of action of the HBsAg/PPLNP formulation. Copied with permission [134]. Copyright 2024, Springer Nature. (C) The structure of PNP/L-PBAE NPs. Reproduced with permission [135]. Copyright 2020, Wiley-VCH. (D) The structure of LPHFNPs@siGFP. Reproduced with permission [136]. Copyright 2025, American Chemical Society.

hanced antigen presentation and immune protection against vaccinia virus challenge [130].

Collectively, lipopeptide-based vaccines provide a promising self-adjuvanting strategy through receptor binding and self-assembly into ordered nanostructures. Minor alterations in lipid chain length, saturation, or linkage site can profoundly influence assembly behavior, receptor affinity, and Th1/Th2 polarization, emphasizing the importance of rational, structure-activity-guided design.

5.2. Lipid-polymer hybrid based antiviral vaccines

Lipid-polymer hybrid nanoparticles (LPHNPs), comprising lipid shells and polymeric cores, combine the mechanical stability and controlled release of polymeric systems with the fusogenicity and biocompatibility of liposomes. The polymeric core protects nucleic acids or proteins from degradation, while the lipid layer enhances cell uptake and endosomal escape [131].

Evidence suggests that the lipid coating surrounding poly (lactic-*co*-glycolic acid) (PLGA) cores can impart muco-inert properties to these hybrid systems, thereby aiding the passage of nucleic acid cargo across mucus-covered human airway epithelial barriers [132]. Due to the neutral nature of PLGA, it is often combined with cationic lipids for mRNA encapsulation. Zhang *et al.* designed a ready-to-use mRNA vaccine formulation (PCDD) by integrating DOTAP, DOPE, cholesterol, and PLGA into hybrid nanoparticles (Fig. 6A). PCDD loaded mRNA encoding an H1N1 HA-M2e fusion antigen, inducing both antibody and T-cell responses in mice [133]. In another approach, a novel polymer-lipid hybrid nanoparticle was constructed using FDA-approved PEG-*b*-PLLA (polyethylene glycol-block-poly(L-lactic acid)) and DOTAP as an adjuvant platform, enabling efficient delivery of the TLR3 agonist poly I:C alongside immobilized hepatitis B surface antigen (HBsAg) (Fig. 6B). This HBsAg/PPLNP formulation significantly promoted antigen uptake and poly I:C delivery, leading to enhanced type I interferon production, improved antigen retention and lymph node trafficking, and robust Th1-skewed immune responses, as evidenced by elevated antigen-specific antibodies, cytotoxic T cell activity, and cytokine secretion [134]. In another study, Li *et al.* synthesized lipid-modified poly(β-amino ester)s (L-PBAEs) *via* enzyme-catalyzed esterification and subsequently co-assembled them with PLGA-PEG to generate "particles-in-particles" (PNP) nanostructures for gene delivery (Fig. 6C). Among the candidates, PNP/C12-PBAE nanoparticles were identified as optimal carriers for DNA and mRNA delivery both *in vitro* and *in vivo*, exhibiting superior transfection efficiency, sustained gene release kinetics, and excellent stability. Notably, these nanoparticles retained their transfection capabilities after lyophilization and storage at −20 °C for up to 12 months [135]. Cabibbo *et al.* further advanced pulmonary delivery strategies by engineering lipid-polymer hybrid nanoparticles for siRNA transport. This platform was built using a novel copolymer derived from α, β-poly(*N*-2-hydroxyethyl)-D,L-aspartamide, which was functionalized with 35 mol% 1,2-bis(3-aminopropylamino) ethane, 0.4 mol% fluorescent tracer, and 4.5 mol% PLGA to encapsulate GFP-targeting siRNA (siGFP). The lipid shell was composed of 1,2-dipalmitoyl-*sn*–glycero-3-phosphocholine and DSPE-PEG_{2000}. The resulting siRNA-loaded lipid-polymer hybrid nanoparticles (LPHFNPs@siGFP) displayed favorable colloidal properties including a hydrodynamic diameter of 164 nm, positive zeta potential, 99% encapsulation efficiency, and a distinct core-shell structure. These nanoparticles achieved efficient cellular uptake and resulted in ~50% silencing of GFP expression in human lung carcinoma cells [136].

Compared with LNPs or single polymer nanoparticles, LPHNPs prevent drug leakage, prolong circulation, and enhance intracellular escape while avoiding burst release, yielding up to fourfold higher transfection efficiency [137]. They also demonstrate superior immune responses, stability, and safety. Nonetheless, their com-

plex core-shell architecture poses challenges for large-scale manufacturing, reproducibility, and pharmacokinetic consistency, which remain critical barriers to clinical translation.

5.3. Glycolipid adjuvant

Lipopolysaccharide (LPS) activates immune cells *via* TLR4 but is too toxic for human vaccines; thus, chemical modification is required. Monophosphoryl lipid A, a detoxified LPS derivative, retains strong immunostimulatory activity with reduced toxicity, making it widely used in vaccines and immunotherapies. AS04, a commercial adjuvant combining MPL with aluminum hydroxide, enhances TLR4 activation and has been approved for HPV and HBV vaccines. Compared with aluminum salts, AS04 elicits stronger humoral and Th1-biased cellular responses [138]. In addition, some other glycolipids, such as glycolipid-α-galactosylceramide (aGalCer), show better adjuvant effects on HIV and malaria vaccines [139]. In addition to MPL/AS04 and α-GalCer, several other glycolipid-based adjuvants have progressed into clinical development or advanced preclinical testing, providing instructive case studies for mechanism and translation. Synthetic glycolipid agonists like glucopyranosyl lipid A (GLA) have entered clinical evaluation. In a phase 1 trial, the tuberculosis vaccine candidate ID93 combined with GLA-SE induced higher ID93-specific antibody titers, increased IgG1/IgG3 levels, and expanded multifunctional $CD4^+$ T-cell responses compared with ID93 alone [140].

Despite these advances, several challenges remain. Excessive TLR4 or iNKT activation may raise safety concerns, and many glycolipid adjuvants rely on complex formulations (*e.g.*, QS-21 liposomes, synthetic LPS emulsions), complicating large-scale manufacturing and batch consistency.

6. Future directions

Despite their transformative impact, lipid-based adjuvants still face challenges in safety, targeting specificity, and dosing. Safety concerns stem from the intrinsic immunostimulatory properties of lipid components. Ionizable lipids can trigger systemic cytokine release (*e.g.*, IL-6, TNF-α) and flu-like symptoms, while cationic lipid accumulation in the liver may cause hepatotoxicity and elevated transaminases, highlighting the need for safer biodegradable analogs [141]. Targeting specificity is another limitation, as apolipoprotein E-mediated uptake directs most LNPs to the liver, restricting delivery to immune tissues such as the spleen or lymph nodes. Moreover, inefficient endosomal escape reduces antigen availability and vaccine efficacy. Although rational design has improved extrahepatic delivery, tissue-specific targeting often requires complex surface engineering or localized administration. Additionally, multiple doses are usually required to achieve durable immunity due to limited antigen retention, transient mRNA expression, and suboptimal memory activation, which can increase toxicity risks and reduce compliance.

6.1. Organelle and tissue-specific targeting

Rationally engineered lipid-based systems enable organ and tissue-specific delivery, enhancing vaccine efficacy, safety, and precision [142]. By directing immunogens to immunologically relevant sites such as the lung, spleen, liver, or CNS, localized immune responses can be strengthened. For instance, mucoadhesive or pH-responsive lipid formulations administered intranasally promote antigen retention and uptake by airway macrophages and DCs, eliciting strong mucosal immunity. Reus *et al.* [143] developed targeted liposomes (LipNaf) modified with apolipoprotein B-derived peptide (ApoB-P) as a specific lung-targeting ligand. Studies demonstrated that this liposomal formulation possessed favorable physicochemical properties, including a nanoscale size (119–147 nm) and a neutral surface charge. The experimental results indicated that the targeted LipNaf exhibited a trend towards reduced viral load in the lungs of SARS-CoV-2-infected mice, with no observable side effects. Lv *et al.* [144] designed a biodegradable ketoester lipid with reduced hepatic tropism and enhanced spleen targeting, yielding stronger mRNA vaccine responses than MC3 or SM-102. Similarly, lymph node targeting can be improved through albumin-binding or mannose ligands, as shown by Dasari's AMP-CpG system [145]. For hepatotropic viruses such as HBV or HCV, ionizable LNPs efficiently deliver mRNA to hepatocytes [146]. Although the CNS remains difficult to access, RVG-modified lipid vesicles successfully delivered mRNA to neurons and reduced Zika-induced neuroinflammation [147].

6.2. Toward long-acting, single-dose, pulsatile vaccines

Sustained antigen exposure (>21 days) is essential for germinal center reactions, high-affinity antibody maturation, and memory formation. However, conventional vaccines have short antigen half-lives (<72 h), requiring boosters. Lipid-based adjuvant systems can achieve long-acting immunization *via* lipid depots or polymer–lipid hybrids that sustain antigen release for weeks or months.

Lipid depots or hybrid systems incorporating phase-transition lipids and polymeric cores (*e.g.*, PLGA-lipid hybrids) can achieve sustained antigen release over weeks to months. Yang *et al.* [148] engineered a nanocomposite adjuvant, termed MIL@A-SW01-C, by integrating polyacrylic acid-coated metal-organic framework MIL-53 (Al) (MIL@A) with a squalene-based O/W emulsion (SW01). MIL@A-SW01-C has good biocompatibility, high antigen loading capacity and continuous antigen release. Compared with conventional adjuvants such as aluminum salts and ISA 201, vaccines formulated with MIL@A-SW01-C elicited significantly elevated antigen-specific serum antibody titers, promoted splenocyte proliferation and cytokine production, and induced a more balanced Th1/Th2 immune response profile. In murine challenge studies, a single immunization with this adjuvant achieved a survival rate of 86.7%. Furthermore, when administered to pigs followed by challenge with wild-type PRV, a single dose of the MIL@A-SW01-C-based vaccine conferred complete protection (100%), markedly surpassing the protective efficacy offered by commercial adjuvant formulations.

6.3. Co-delivery of multiple immune-modulatory components

Co-delivery of antigens and immune-modulatory agents within a single lipid nanoparticle ensures synchronized delivery to APCs and coordinated immune activation. Lipid-based carriers provide a versatile platform for co-delivery, allowing simultaneous encapsulation of nucleic acid antigens (*e.g.*, mRNA, DNA), protein or peptide epitopes, and a wide array of immunostimulatory molecules. Notably, the co-delivery of antigen and pattern recognition receptor agonists-such as TLR or STING agonists-has shown superior immunogenicity compared to antigen alone. For example, mRNA-LNPs co-loaded with TLR7/8 agonists or STING agonists (*e.g.*, cyclic dinucleotides) have been demonstrated to induce robust DC maturation, type I interferon responses, and Th1-skewed adaptive immunity in preclinical models of viral infection and cancer. This co-delivery not only enhances antigen presentation *via* MHC class I and II pathways but also promotes the recruitment and activation of $CD8^+$ CTLs and $CD4^+$ helper T cells. Additionally, co-delivery enables combination adjuvant strategies that mimic natural infection cues more effectively. Emulsion-based adjuvants such as AS01, for instance, incorporate both MPL and QS-21, which synergize to activate different signaling cascades, resulting in enhanced germi-

nal center formation, antibody affinity maturation, and long-lived memory responses.

6.4. Multivalent vaccines

The lipid-based vaccine system provides a reasonable framework for multivalent preparations. Lipid-based vaccines can simultaneously encode or encapsulate multiple viral antigens, thereby expanding immune coverage and reducing the impact of antigenic variation. Recent studies have included tetravalent or hexavalent mRNA-LNP pharmaceutical preparations for different influenza HA subtypes, which have induced cross-protective responses to mismatched strains in animal challenge studies [149]. Similarly, the multivalent LNP vaccine combined with the SARS-CoV-2 mutant spike protein showed a stronger breadth advantage than the monovalent preparation in terms of neutralization [150]. The synergistic delivery ability of the lipid-based system ensures the simultaneous display of multiple antigens in the same APC, reduces immune interference, and improves protection persistence. These characteristics indicate that the LNP-based adjuvant platform can accelerate the clinical application of multivalent vaccines (group protection against diverse or rapidly evolving viral threats).

7. Manufacturing, regulatory and broader challenges

7.1. Fabrication

Scalable manufacturing of lipid-based adjuvants remains challenging. Techniques such as ethanol injection and microfluidics improve liposome uniformity but face issues of reproducibility, efficiency, and cost [151,152]. Industrial-scale LNP production using film hydration, extrusion, homogenization, or T-junction mixing requires optimization of encapsulation efficiency and particle uniformity [153]. Similarly, MF59 emulsions rely on microfluidization for precise droplet control but involve high equipment costs and operational complexity [65].

7.2. Quality control

Quality assurance for lipid-based adjuvants represents a critical pharmaceutical imperative, particularly in liposomal vaccine development and therapeutic liposome production, to ensure product safety, efficacy, and stability. Ensuring consistent particle size ($<$100 nm), drug retention, near-neutral zeta potential, and stability is essential for liposomal and LNP vaccines [154]. For mRNA-LNPs, key quality attributes include particle size (60–150 nm), encapsulation efficiency (assessed *via* RiboGreen), and apparent pK_a [155]. Emulsion-based adjuvants like MF59 also demand strict control of particle size ($\sim$160 nm), viscosity, and oil-to-water ratio.

7.3. Additional considerations

Intellectual property, technology transfer, and regulatory harmonization influence global vaccine access. Expedited regulatory pathways, such as Health Canada's rolling review during COVID-19, can accelerate deployment without compromising safety. Cold-chain logistics remain critical, as vaccines must be transported under validated temperature conditions [156,157]. Batch-to-batch variability in emulsions or liposomes and differing regulatory requirements across regions (*e.g.*, delayed MF59 approval in the U.S.) complicate global rollout. Strengthening IP governance, manufacturing capacity, and regulatory coordination will be key to building a resilient global vaccine ecosystem.

8. Conclusion and outlook

The continuous emergence of viral epidemics and pandemics has underscored the urgent need for vaccine platforms that are not only effective and safe, but also adaptable, scalable, and capable of inducing broad and durable immune responses. Lipid-based delivery systems have rapidly emerged as a transformative technology in this context, enabling the successful development and deployment of nucleic acid vaccines at an unprecedented speed and scale.

From liposomes and oil-in-water emulsions to LNPs, these systems provide versatile platforms that can encapsulate and protect diverse antigens, enhance the uptake of APCs, modulate immune responses through co-delivered adjuvants, and be tailored for tissue-specific or mucosal delivery. Their physicochemical properties, modular design, and clinical track record offer significant advantages over polymeric, viral, and inorganic vectors, especially in terms of biocompatibility, manufacturability, and regulatory acceptance. Nevertheless, antigenic diversity and rapid viral evolution demand next-generation lipid systems capable of supporting multivalent and variant-proof vaccine designs. Tissue targeting and mucosal immunity require further innovations in formulation and administration routes. Additionally, large-scale manufacturing, cold-chain logistics, and equitable global distribution must be addressed in tandem with scientific advances to ensure that lipid-based vaccines can fulfill their global public health potential.

In summary, lipid-based vaccines hold significant promise for the prevention and management of epidemic viral infections. Continuous exploration and innovation in lipid carriers are crucial to unlocking their full potential in addressing the challenges posed by viral epidemics and rapid evolution. Lipid-based vaccine platforms will remain at the forefront of vaccinology, enabling rapid containment of emerging viral threats and fundamentally enhancing our capacity to prevent and control infectious diseases.

CRediT authorship contribution statement

Yan-Qi Zhao: Writing – original draft, Conceptualization. **Zi-Jian Cheng:** Writing – original draft. **Dian Cai:** Writing – original draft. **Yue-Lin Fang:** Writing – original draft. **Jian Xu:** Writing – original draft. **Hong-Lan Dai:** Writing – original draft. **Wen-Qi Liu:** Writing – original draft. **Xin-Xin Zhang:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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