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Influence of structural modifications in synthetic vectors of lipid adjuvants on mRNA vaccine delivery

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Lipid adjuvants act as a fundamental element in mRNA vaccine technology by performing as diverse functional parts: augmenting immune responses, assisting genetic payload delivery to target cells, and optimizing antigen presentation. They offer various advantages, such as particle stabilization, targeted delivery, refined endosomal escape mechanisms, and self-adjuvant characteristics that amplify immune activation. The lipid adjuvant structure is crucial for both maximizing delivery accuracy and unlocking tunable immune responses, positioning lipid adjuvants as critical components of next-generation vaccines. Understanding the structural alterations of the lipid adjuvants is necessary for the rational design and synthesis of next-generation novel lipid adjuvants that elicit superior immune responses in mRNA vaccines. To magnify the potency and safety of lipid adjuvants, researchers are investigating the fundamental aspects of designing an innovative lipid that leverages biodegradable linkages. This strategy emphasizes the critical roles of numerous lipids, such as ionizable/cationic lipids, helper lipids, phospholipids, and PEGylated lipids, for enhancing the stability, targeting precision, and immunogenic efficacy of mRNA vaccine delivery. Moreover, it elucidates the structural changes of recently developed cationic/ionizable lipid adjuvants, highlighting how their structure impacts vaccine efficacy, especially linkers. By leveraging these advancements, researchers are exploring the potential for highly effective and targeted mRNA vaccine platforms, paving the way for next-generation immunization strategies.

1. Introduction

An adjuvant is an additive that is added to antigens in vaccinations to stimulate the immune response. It is a crucial part of contemporary vaccines because it augments immunogenicity, diminishes the need for antigens, boosts humoral and cell-mediated immunity, improves responses in vulnerable groups, and ultimately leads to more successful vaccination campaigns.[1,2] Aluminum hydroxide [$Al(OH)_3$], a widely utilized adjuvant in human vaccines, predominantly induces Th2 immune responses. While this Th2 bias is advantageous for eliciting strong humoral immunity, it limits the efficacy of aluminum hydroxide against pathogens that require robust Th1-mediated cellular responses, thereby underscoring the need for the development of adjuvants capable of generating more specialized and balanced immune profiles.[3] Traditional adjuvants, such as aluminum hydroxide, are associated with several limitations, including the potential for long-term bio persistence and neuroinflammation in the brain, suboptimal induction of cell-mediated (Th1) immunity, and incomplete understanding of their mechanisms of action. These drawbacks may result in inadequate long-term immunity, increased risk of adverse events, and restricted efficacy in generating balanced Th1/Th2 responses, which are essential for optimal humoral and cellular immune function.[4] Lipid adjuvants in disease-specific vaccines enable precise modulation of immune responses, enhancing protective immunity while minimizing adverse effects such as hypersensitivity and autoimmunity. In mRNA vaccine platforms, lipid-based delivery vectors not only facilitate efficient antigen and mRNA delivery to antigen-presenting cells (APCs) but also serve as intrinsic immunostimulatory ligands, amplifying immune activation. The integration of adjuvant functionality directly into ionizable lipid nanoparticle (LNP) architectures, rather than as separate additives, introduces complexity in adjuvant selection and optimization for specific disease targets, posing a significant challenge for vaccine development. Nevertheless, lipid-based adjuvants, particularly those employing lipid nano-

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particles (LNPs) in the newly developed mRNA vaccine technology, have demonstrated significant advantages over traditional vaccine adjuvants. These advantages are characterized by high efficacy, accelerated advancement, effective administration, reduced manufacturing costs, targeting capabilities and desirable therapeutic applications.[5,6]

Lipid adjuvants are synthetically engineered in the laboratory with structural and functional attributes tailored to the immunological requirements of specific disease targets. These adjuvants are typically formulated into vesicular assemblies with auxiliary lipids, forming LNPs that facilitate the efficient delivery of mRNA vaccines. The molecular architecture of cationic or ionizable lipid adjuvants is critical for optimizing mRNA encapsulation, cellular uptake, and endosomal escape. Effective nucleic acid delivery necessitates the careful rational design of lipid components, considering key parameters such as head group ionizability, hydrophobic tail composition, linker chemistry, and overall physicochemical properties. Additionally, mechanistic considerations—particularly those governing endosomal destabilization and cytosolic release—are essential to ensure robust intracellular distribution and transgene expression.[7] In 1987, Philip Felgner pioneered the design of *N*-[1-(2,3-dioleyloxy)propyl]-*N*,*N*,*N*-trimethyl ammonium chloride (DOTMA), the first synthetic cationic lipid adjuvant for nucleic acid delivery, marking a foundational advance in the field. Subsequent decades have seen extensive structural refinement of lipid adjuvants and nanoparticle formulations, with modifications primarily targeting features that critically influence packing morphology and particle stability—namely, the hydrophobic domain, the chemical nature of the charged head group, and the type of chemical linkage connecting these domains. These rational modifications have enabled enhanced nucleic acid encapsulation, improved cellular uptake, and more efficient endosomal escape, thereby optimizing the performance of delivery systems based on lipids in gene and mRNA vaccine applications.[8] Establishing direct correlations between the structural attributes of lipid adjuvants and their transfection efficacy remains challenging, as structure–activity relationships are often complex and multifactorial. Despite the identification of certain general trends—such as the enhanced performance associated with double-chain hydrophobic domains and the substitution of monounsaturated for saturated lipid tails—optimization strategies for lipid adjuvant systems largely rely on empirical approaches. These structural considerations are critical for the continued advancement of non-viral lipid-based delivery platforms for mRNA vaccines, with significant implications for their therapeutic potential and clinical translation.[9] The structural and functional properties of LNPs result from the combined effects of multiple lipid components. This review explores the structure and functional relationships of novel synthetic lipid adjuvants in mRNA vaccines, focusing on rational lipid design to maximize efficacy and minimize side effects. We analyze how structural features—such as linker chemistry, hydrophobic tails, and head groups—influence LNP performance, mRNA delivery, and immunogenicity. This review aims to delve into the intricate relationship between the recently developed lipid adjuvant structure and its function in mRNA vaccines. By providing information on the rational design of lipid adjuvant chemistry for next-generation mRNA delivery systems, we can maximize therapeutic potential while minimizing adverse effects and better understand the underlying mechanism. We explore the pivotal roles of various lipids in vaccine formulations, with a particular focus on synthetic lipid adjuvants. By examining how the structure of these adjuvants, especially linkers, influences the function of specific components such as hydrophobic tails and head groups, we uncover the mechanisms behind their impact on mRNA

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vaccine delivery and immune responses. By clarifying how these modifications affect key processes like encapsulation, uptake, endosomal escape, and immune activation, we propose design principles to guide the development of safer, more effective, and targeted lipid adjuvants for next-generation mRNA vaccine delivery systems.

2. Lipid nanoparticles as adjuvants: mechanism, structural variation impact on transfection, and lipid components in LNPs

LNPs are carrier vectors of messenger RNA in mRNA vaccine technology. Liposomes are the ancient version of LNPs, tiny-globe-shaped structures with lipid bilayers.[10] The vaccine adjuvant in LNPs was demonstrated by Allison and Gregoriadis in the year 1974. Researchers demonstrated that LNPs, which were formulated with phospholipids and the diphtheria toxin (DT), could enhance the immune responses of an antigen used in vaccines to protect against diphtheria.[4] Researchers could increase the safety, reliability, and efficacy of LNP formulations, which could improve immune responses and vaccine performances, by tailoring the structural characteristics of the lipids. Smaller LNPs (around 100 nm) favor Th2 responses conducive to antibody production, while larger LNPs (400 nm or more) promote Th1 responses that enhance cell-mediated immunity in the case of liposome vaccine delivery for leishmaniasis and malaria.[10–13]

LNPs have emerged as pivotal components in modern vaccine development, performing multiple critical functions. They act as sophisticated carriers, efficiently encapsulating both immune-stimulatory adjuvants and antigens—often in the form of mRNA—thereby ensuring targeted delivery and enhanced cellular uptake. Remarkably, LNPs also possess intrinsic self-adjuvant properties, attributed to their unique lipid constituents, which further amplify immune responses. Each lipid in a lipid formulation plays a specific role. These formulations consist of four major parts, namely cationic lipids or ionizable lipids, phosphorus lipids, steroidal lipids (cholesterol variants), and PEG (polyethylene glycol) lipids, as depicted in Fig. 1. Cationic or ionizable lipids are synthetic lipid adjuvants that have a positive or neutral head group domain with a hydrophobic tail; both are attached through specific linkers like ethers, esters, triazoles, secondary amines, S–S bonds, carbamides, β-amino alcohols, amides, *etc.*[14,15] Phospholipids function as helper lipids within LNP formulations, contributing to structural stabilization and mimicking the properties of biological membranes. Cholesterol serves as an additional structural component, modulating the rigidity, stability, and fluidity of the LNP bilayer. PEG lipids create a hydrophilic "stealth" shield on the nanoparticle surface, enhancing the circulation time by protecting LNPs.[16] The productivity of LNPs in the evaluation of mRNA vaccines is ascribed not just to individual components but to the synergistic effects of various lipids within the formulation. So, it is

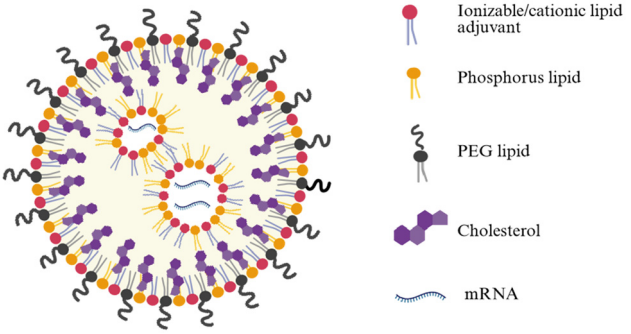

Fig. 1 Structure and compositional elements of the lipid nanoparticles: an ionizable/cationic lipid adjuvant, phosphorus lipid, PEG-lipid, cholesterol, and mRNA.

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important to know about each moiety used in lipid nanoparticle formulations and their working methods.[17]

2.1. Mechanism of action

By protecting mRNA from degradation, facilitating endocytosis, and improving the delivery of mRNA to cells, LNPs are recognized and internalized by antigen-presenting cells (APCs) through receptor-mediated endocytosis or phagocytosis. As a result, the efficient presentation of associated antigens to the immune system is enabled, promoting antigen presentation and the subsequent activation of T cells. LNPs activate innate immune responses by engaging TLRs (*e.g.*, TLR4, TLR7, and TLR9), which then drives the secretion of proinflammatory cytokines and chemokines.[18] By strengthening the presentation and distribution of antigens to APCs, LNPs offer a potential approach for vaccine development that will ultimately result in more powerful and efficient immune system responses and can further be significantly improved through the strategic engagement of pattern recognition receptors (PRRs) and pathogen-associated molecular patterns (PAMPs). Following intramuscular injection, a depot is established by LNPs at the injection site, from which they subsequently migrate to the draining lymph nodes, thereby facilitating efficient antigen delivery. This process allows antigen-presenting cells in the lymph nodes to capture and process the antigens. Small nanoparticles can indeed drain freely to lymph nodes, where they are taken up by resident dendritic cells (DCs) and macrophages. In contrast, larger particles (over 500 nm) primarily rely on cellular transport by tissue-specific DCs to reach the lymph nodes. The size of particles significantly influences their trafficking mechanisms to lymph nodes.[19,20]

The encapsulation of antigens in the form of mRNA by LNPs is a major component in vaccine development, serving dual roles as carriers for both immunostimulatory adjuvants and antigens, which are encoded by mRNA, while also exhibiting self-adjuvant properties due to their lipid components. Lipid adjuvants deliver the antigens to APCs, significantly increasing APC activation and function, which upregulates co-stimulatory molecules such as CD40, CD80, and CD86 and antigen presentation signals. Major histocompatibility molecules are used by APCs to process the antigens and present them on the surface of APCs. Cytotoxic T lymphocytes are presented with antigens through major histocompatibility molecules, which improves cytotoxic T cell engagement and boosts the effectiveness of the immune response, which leads to T cell differentiation, proliferation, and memory cell formation. High-density antigen presentation *via* LNPs can further enhance B cell activation through direct engagement with B cell receptors (BCRs) and boost BCR crossing, leading to immune responses.[21] B cell activation depends on the interaction between CD40 on B cells and CD40L on activated T helper (Th) cells. This interaction sets off a sequence of intracellular signaling cascades that encourage the growth of B cells, the switching of immunoglobulin isotypes, and the development of plasma cells that can produce antibodies against particular antigens. These mechanisms collectively contribute to a robust adaptive immune response, as shown in Fig. 2.[22]

2.2. Do structural variations of ionizable/cationic lipid adjuvants really affect the efficacies and adjuvant properties of mRNA vaccine delivery?

Liposomal nanoparticle or LNP adjuvanticity is greatly influenced by ionizable/cationic lipid adjuvants. The fluidity of liposomal bilayers is influenced by their composition and structure. The physical characteristics of LNPs are influenced by variables such as double bond locations, hydrocarbon chain lengths, and saturation. Modifications such as acyl chain length adjustments (*e.g.*, shorter chains at lower temperatures) influence lipid stability and its ability to bind immune receptors like TLR4. Due to their capacity to create a

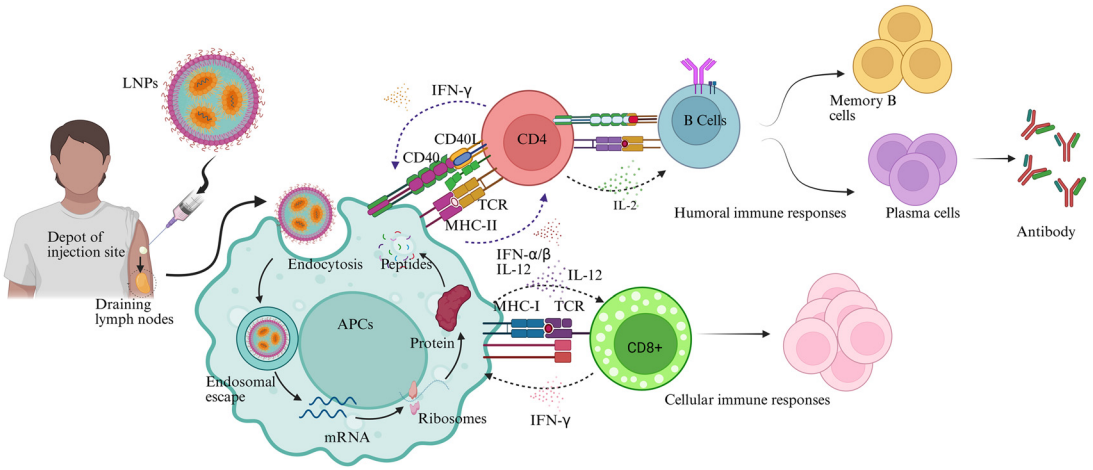

Fig. 2 Mechanism of LNP-mRNA vaccines: lipid adjuvants involved in the activation of antigen-presenting cells (APCs), which in turn stimulate humoral (B cell) and cellular (T cell) immune responses.

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depot at the injection site and consistently draw APCs, rigid liposomes made with a saturated analog such as dimethyldioctadecylammonium (DDA) elicit greater immune responses, including higher Th1 responses, than more fluid liposomes made with an unsaturated analog, such as dimethyldioleoylammonium (DODA). This rigidity of the liposome comes from the structure of DDA, which has two long chains, whereas DODA has one double bond.[23,24] Inflexal®V and Epaxal vaccines use 1,2-dioleoyl-*sn-glycero*-3-phosphocholine (DOPC) and dioleoyl-*sn-glycero*-3-phosphoethanolamine (DOPE) lipids. 1,2-DOPE promotes a non-lamellar framework, improving fluidity and transfection accuracy. DOPC reduces fusogenicity and forms enduring lamellar structures, improving membrane mobility and preserving structural integrity.[25] The proportion of DOPC to DOPE in vaccines like Epaxal® creates a stable, dynamic barrier, enhancing immune responses and ensuring the stability, fluidity, and efficacy of these vaccines, which supports their efficacy as virosomal adjuvanted vaccines.[26]

For example, *N*-[1-(2,3-dioleyloxy)propyl]-*N*,*N*,*N*-trimethyl ammonium chloride (DOTMA) and 1,2-dioleoyl-3-trimethylammonium propane (DOTAP) are considered to have highly similar structures, differing primarily in their linker moieties. However, very dissimilar functions are exhibited by these lipids upon cellular entry. Compared to DOTMA, DOTAP is easily degradable because of the ester linker and delivers mRNA into the cells, leading to different immune responses as elaborated below. In this article, the variation of immune responses as influenced by the structure of lipid adjuvants is emphasized. As lipids are composed of multiple components, the safe and efficient packaging and distribution of nucleic acid cargos are determined by the precise construction of each component.[27] Intensified focus is being placed on the specific importance of each lipid component in transfection studies by researchers. For the design of lipid adjuvants for next-generation vaccines, understanding both the overall characteristics of each LNP component and specific lipid structure could prove beneficial. By manipulating lipid composition and structure, researchers can design more effective adjuvants that optimize immune responses tailored to specific pathogens or therapeutic needs. Understanding these relationships is essential for advancing lipid adjuvant-based vaccine development for improving public health outcomes. Various lipid types with lipid adjuvants have been used in LNP formulations as depicted in Fig. 3.

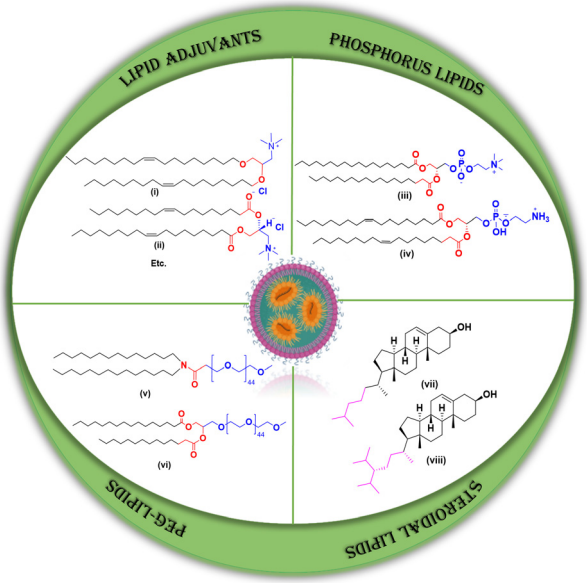

Fig. 3 The types of lipids contained in the LNP formulations, (i) DOTMA, (ii) (*R*)-DOTAP, (iii) 1,2-distearoyl-*sn-glycero*-3-phosphocholine (DSPC), (iv) DOPE, (v) (4-hydroxybutyl)azanediylbis(hexane-6,1-diyl)bis (2-hexyldecanoate)(ALC-0159), (vi) 1,2-dimyristoyl-*rac-glycero*-3-methoxypolyethylene glycol-2000(PEG-DMG-2000), (vii) cholesterol, and (viii) β-sitosterol.

2.3. Role of steroidal lipids for immune responses

Cholesterol enhances the structural integrity of lipid nanoparticles by modulating membrane fluidity and rigidity, thereby optimizing the stability of the lipid bilayer. An increased molar ratio of cholesterol facilitates a critical lamellar-to-hexagonal phase transition, which is essential for destabilizing the endosomal membrane and promoting the efficient release of encapsulated mRNA into the cytosol. This phase transition is a key mechanistic step in improving the endosomal escape and overall delivery efficiency of mRNA-loaded lipid nanoparticles.[18] Cholesterol is a critical component for the cryoprotection of LNPs during lyophilization, as it stabilizes the lipid bilayer and prevents aggregation and nucleic acid leakage.[28] The cholesterol-induced phase (ChiP) is characterized by the absence of crystalline cholesterol forms and may potentially be linked to the adjuvant activity of LNPs.[29] Cholesterol incorporated within LNPs is efficiently metabolized by macrophages into oxysterols, which function as potent immunoregulatory molecules. This metabolic conversion underscores the necessity of elucidating the *in vivo* fate of LNP-derived cholesterol and delineating the mechanistic pathways that connect cholesterol metabolism with the modulation of immune responses.[30]

β-Sitosterol, a predominant plant sterol found in the human diet, exhibits multiple medicinal properties, like antioxidant and anti-cancer activities, anti-inflammatory effects and modulation of lipid metabolism.[30] Incorporation of β-sitosterol into LNP formulations significantly enhances mRNA transfection efficiency by altering nanoparticle surface composition and morphology, often promoting a polyhedral structure that facilitates improved membrane fusion and endosomal escape. These structural changes enhance the delivery of the cargo mRNA to the immune cells, eventually leading to improved T-cell proliferation, elevated IL-2 production, and more efficient transfer of the mRNA cargo to target immune populations.[31,32]

2.4. Role of phosphorus lipids for immune responses

In mRNA vaccination therapy, adjustment of phospholipid composition enhances LNP administration effectiveness, stability, endosomal escape, toxicity, and organ targeting.[33]

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Because they produce lipid bilayers and preserve membrane fluidity, phospholipids are crucial for immunological responses and cellular membranes. Enzymatic control of metabolism is crucial for the proper generation of lipid mediators in immune responses. Comprehending these roles offers valuable perspectives on possible treatment methods for regulating immune reactions in illnesses. The function of phospholipids in phagocytosis is essential.[34] Notable lipids found in liposomal formulations of mRNA lipid adjuvant vaccines are DSPC, DOPC, and DOPE. The hexagonal shape of DOPE promotes membrane fusion and cellular uptake.[9] On the other hand, DOPE and DOTMA cause unfavorable human inflammatory reactions by inducing reactive oxygen species in monocyte mitochondria. The adjuvant properties of DOPE emphasize its function in immune activation.[35,36] It is noteworthy that, in comparison with DSPC-containing LNPs, DOPE-containing LNPs demonstrated a significantly improved ability to escape from endosomes.[33]

Phospholipids like phosphatidylcholine (PC) and phosphatidylethanolamine (PE) are key to improving LNP performance. While PC boosts cellular uptake, it doesn't always enhance intracellular delivery. Unsaturated lipids such as DOPC and 1-stearoyl-2-oleoyl-*sn-glycero*-3-phosphocholine (SOPC) promote membrane fusion and endosomal escape, leading to better uptake and intracellular distribution for gene delivery. Thus, phospholipid composition is crucial for optimizing LNP-based delivery systems.[37]

2.5. Role of PEG-lipids for immune responses

PEG is crucial in LNP formulations for its stealth properties, forming a hydrophilic layer that reduces protein adsorption and immune clearance. This enhances colloidal stability and extends the circulation time. The effectiveness of PEG depends on the length of its lipid anchor and the molecular weight of the PEG chain, with longer anchors and higher PEG density providing better stability and protection.[38] Using a mouse model of traumatic brain injury (TBI), the pharmacokinetics and efficacy of LNPs made with PEG-lipids of various anchoring configurations were assessed when administered systemically.[39] PEG is generally considered nonimmunogenic and safe by the FDA, but recent studies show its immunogenicity is more complex than previously thought. However, it has been indicated by recent studies that PEG's immunogenicity is more complex than was previously believed. To enhance the design and effectiveness of LNPs in lipid-based adjuvants for mRNA vaccines, an understanding of the circumstances in which PEG becomes immunogenic is essential. Anti-PEG antibodies are produced by the immune system in response to the presence of PEG in the body. It is suggested by research that these antigenic determinants are primarily located at the linker regions where PEG is conjugated to other materials, such as proteins or lipids. Recognizing the phase between a hydrophobic core and conjugated PEG groups as an epitope for anti-PEG antibodies highlights its significance in immunogenicity studies and LNP formulation strategies.[40,41]

3. Role of cationic/ionizable lipids in LNPs as adjuvants

The ionizable/cationic lipid is the principal determinant of the adjuvant activity in LNP formulations used for mRNA vaccines, exerting the most pronounced influence on the immunostimulatory properties of the LNPs. While these helper lipids are indispensable for the structural integrity, stability, and biodistribution of LNPs, their contribution to adjuvanticity is secondary to that of the ionizable lipid, which is the primary driver of immune activation in mRNA-LNP vaccines.[29] Researchers have engineered novel target-specific cationic and ionizable lipids tailored to address the therapeutic demands of distinct disease indications. These self-adjuvating lipids potentiate immune responses by augmenting antigen recognition and facilitating a precise, context-dependent activation of the immune system. This review delineates the functional roles and mechanistic contributions of various cationic and ionizable lipid adjuvants incorporated within LNP formulations, emphasizing their impact on immunogenicity and vaccine efficacy.[28] Cationic lipids possess a permanently positive head group linked to a hydrophobic domain, enabling strong electrostatic binding to nucleic acids and efficient transfection, but their constant charge also increases toxicity and rapid clearance. In contrast, ionizable lipids contain amine head groups that are neutral at physiological pH—reducing toxicity—but become positively charged in acidic endosomal environments, promoting endosomal escape and nucleic acid delivery. The structural features of ionizable lipids (head group, linker, and hydrophobic tail) critically affect encapsulation, delivery efficiency, and safety. The clinical success of ionizable lipids like SM-102 (heptadecan-9-yl8-((2-hydroxyethyl)(6-oxo-6-(undecyloxy)hexyl)amino) octanoate), ALC-0315, and DLin-MC3-DMA ((6*Z*,9*Z*,28*Z*,13*Z*)-heptatriactonta-6,9,28,31-tetraen-19-yl-4-(dimethylamno)butanoate) has made them the preferred choice for modern LNP-based nucleic acid delivery systems.[42,43] Thus far, we have delineated the fundamental structural architecture of cationic and ionizable lipids, which are composed of hydrophobic and hydrophilic domains interconnected *via* a linker moiety. The structural attributes of various lipid adjuvants are systematically analyzed and categorized according to their hydrophobic tails, head groups, and linker regions, as illustrated in Fig. 4. The subsequent sections will provide an in-depth evaluation of each structural component, with a particular emphasis on the linker, and discuss how specific modifications within these domains can modulate immunological outcomes by functioning as adjuvants.

3.1. Importance of the hydrophobic domain

Structure–activity relationship (SAR) studies have demonstrated that the non-polar hydrophobic domains of lipids critically influence the physicochemical properties of LNPs, including membrane fluidity, phase transition behavior, colloidal stability, and cytotoxicity. Lipids possessing two hydrophobic

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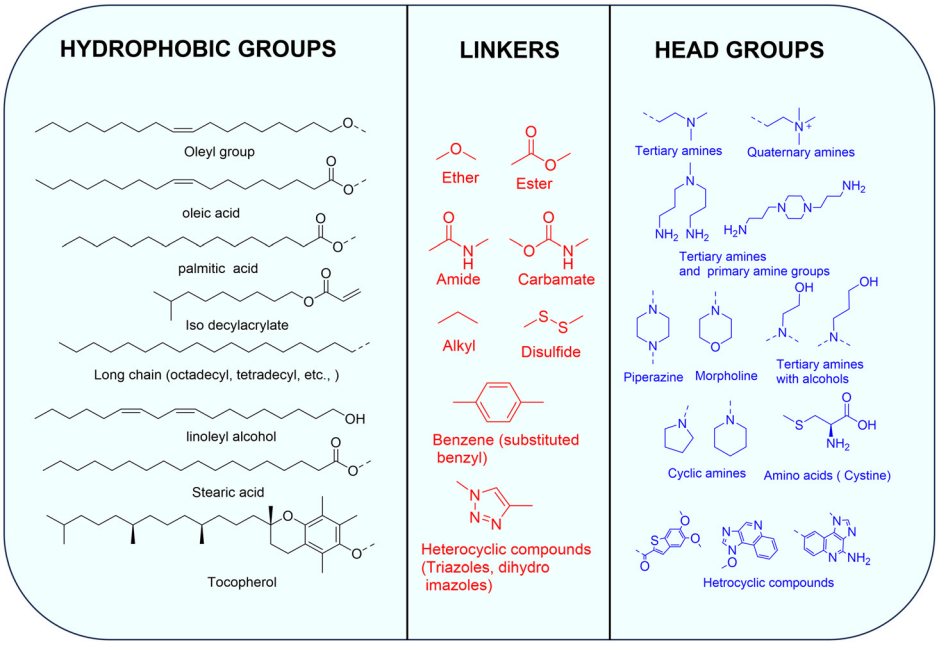

Fig. 4 Chemical structures demonstrate the distinct hydrophobic, hydrophilic, and linker regions within the lipid adjuvants.

tails tend to self-assemble more efficiently in aqueous environments, resulting in enhanced transfection efficiency. These hydrophobic moieties may comprise saturated or unsaturated long-chain hydrocarbons, sterols, tocopherol derivatives, or other extended non-polar structures, each contributing distinctively to the overall performance and biological compatibility of LNP formulations.[27] There is uncertainty over how many aliphatic chains are optimal for cationic lipid transfection. Phase behavior, internal density drops, and assembly stiffness are reduced when two tails are asymmetrical. Lipids like C18:1C10-EPC, which promote nonlamellar phase formation, show higher transfection efficiency. Overall, asymmetric cationic lipids improve cellular uptake, endosomal escape, and gene delivery compared to symmetric ones, highlighting the significance of hydrophobic domain design in optimizing non-viral gene delivery.[9,44] Alkyl tail branching markedly influences the molecular geometry of surfactants and is a critical parameter in the rational design of functional lipids for nucleic acid therapeutics. Additionally, the degree of unsaturation within the hydrophobic tails modulates membrane destabilization properties, thereby affecting the efficiency of nucleic acid delivery. These structural features collectively determine the biophysical behavior of lipid assemblies, impacting their capacity for endosomal escape and intracellular release of genetic cargo.[43,45] Tocopherol (vitamin E) acts as a key hydrophobic component in lipid nanoparticles, supporting both structure and function. It modulates immune responses by affecting cell signaling, membrane dynamics, and the production of inflammatory mediators like cytokines and PGE2. Tocopherol also enhances T cell membrane integrity, signal transduction, and proliferation. Incorporating vitamin E-based cationic lipids improves delivery, immunogenicity, and therapeutic outcomes in mRNA vaccines and gene therapies for cancer and genetic diseases.[46,47] ALC-0315 and SM-102, both employed in mRNA vaccine formulations, are characterized by the presence of two-branched, saturated alkyl tails, which impart a cone-shaped molecular geometry. This structural feature is postulated to enhance endosomal membrane destabilization, thereby facilitating efficient cytosolic release of encapsulated nucleic acids. In contrast, DLin-MC3-DMA contains two linoleoyl tails, which may confer distinct biophysical properties that further promote endosomal escape and enhance the overall efficacy of nucleic acid delivery systems. The differential tail architectures of these ionizable lipids underscore the importance of hydrophobic domain design in optimizing intracellular delivery and therapeutic performance.[48]

3.2. Importance of the hydrophilic domain

The size and charge density of polar head groups are critical determinants of LNP stability, cellular membrane interactions, and nucleic acid condensation efficiency. However, clinical translation of cationic and ionizable lipid-based formulations has been hindered by insufficient evaluation of the significant relationship between polar head group structure and associated toxicity. Notably, lipids incorporating primary, secondary, or tertiary amine head groups generally exhibit lower cytotoxicity compared to those containing quaternary ammonium groups, underscoring the importance of head group selection in the design of safe and effective LNP systems.[9,14] The hydrophilic head group of lipids can comprise a variety of functional moieties, including amines (primary, secondary, tertiary, and quaternary), amino acids, heterocyclic structures (such as pyrazine, piperazine, indole, pyrazole, and purine), carbohydrates,

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and phosphorus-containing groups. The chemical nature of the head group is crucial for mediating interactions with nucleic acids, particularly through electrostatic attraction between the positively charged cationic lipid head groups and the negatively charged phosphate backbone of nucleic acids. This interaction is fundamental for the formation and stabilization of LNPs, ensuring efficient encapsulation and protection of nucleic acids during delivery to APCs. Consequently, the physicochemical properties of the head group significantly influence the overall stability and efficacy of LNP-based delivery systems.[48] Hydrophilic head groups improve lipid adjuvant solubility, while cationic charges boost cellular uptake. The balance of these properties is key to immunostimulatory efficacy. Head group modifications, such as glycosylation, can target specific receptors (*e.g.*, lectins on APCs) to enhance uptake and immune activation. Certain head groups also provide co-stimulatory signals for T-cell activation, supporting long-lasting immunity. Incorporating functional groups into ionizable lipids further enhances immunogenicity and vaccine effectiveness.[49] Clinically used ionizable lipids like DLin-MC3-DMA, SM-102, and ALC-0315 have tertiary amine head groups with pH-dependent ionization, aiding endosomal escape and nucleic acid delivery. SM-102 and ALC-0315 also feature terminal hydroxyl groups that enhance hydrogen bonding with nucleic acids, improving transfection efficiency. The head group structure in these lipids is crucial for immune modulation, nanoparticle stability, antigen presentation, and co-stimulatory signaling. Understanding these interactions at a molecular level is crucial for designing more effective vaccine adjuvants that can significantly improve immunogenicity and therapeutic outcomes.[14]

3.3. Importance of the linker domain

Linkers are critical for maintaining the structural stability of lipids by connecting their polar and nonpolar domains. These linkers encompass a diverse range of chemical groups, including ethers, esters, amides, carbamides, disulfide (S–S) bonds, triazoles, alkyl chains, imidazole rings, β-amino alcohols, *etc*. The incorporation of biodegradable linkages—such as those sensitive to pH, redox conditions, or enzymatic cleavage—offers distinct advantages in terms of lipid efficacy and cytotoxicity, facilitating improved biocompatibility and controlled degradation within biological environments.[14] Ether bonds exhibit high resistance to hydrolytic degradation, whereas ester and amide bonds are readily cleaved by hydrolysis, rendering them biodegradable. Consequently, ester and amide linkages are predominantly employed in the design of cationic and ionizable lipid adjuvants to facilitate controlled degradation and clearance. Disulfide (S–S) bonds, being redox-sensitive, are specifically cleaved under intracellular reducing conditions, enabling the targeted release of encapsulated antigens, nucleic acids, or therapeutics within cells. This strategic use of biodegradable and stimuli-responsive linkers enhances the precision, efficiency, and safety of lipid-based delivery systems.[50] Recent advances in lipid adjuvant design emphasize novel and multifunctional linkers, which are discussed and depicted in Fig. 5 and 6. To improve delivery efficiency and reduce toxicity, strategies such as adding spacers between the

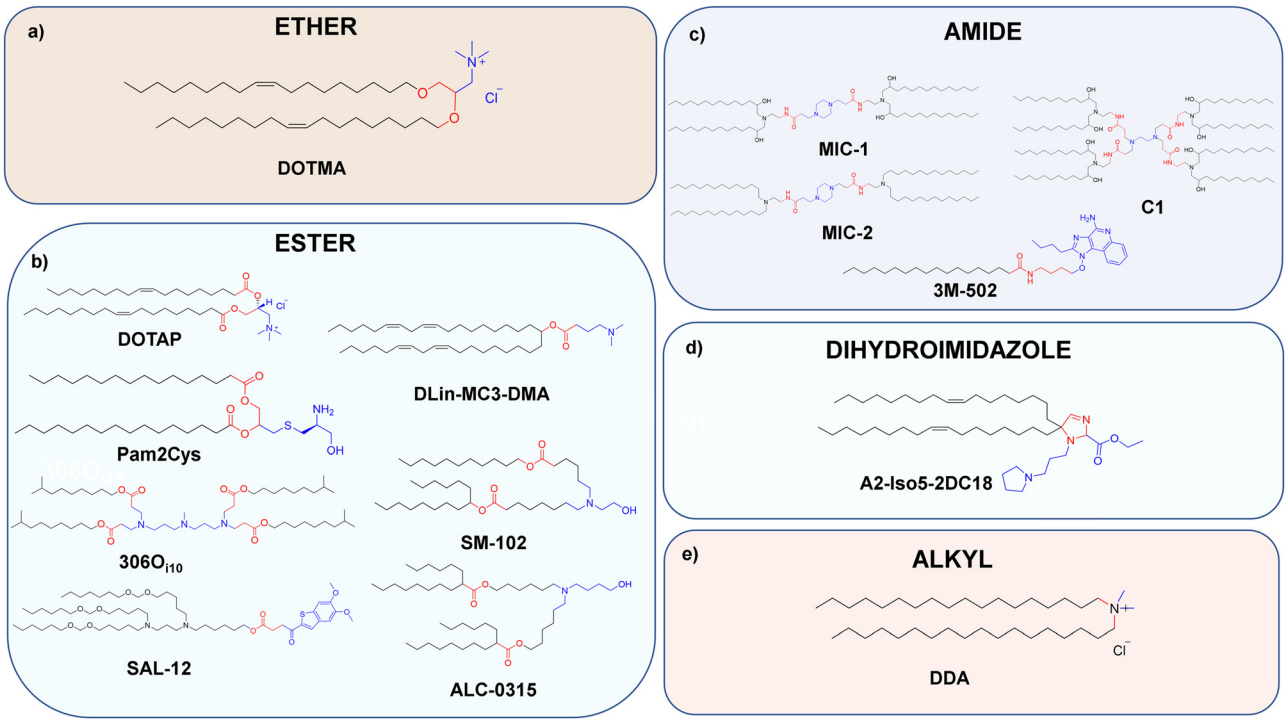

Fig. 5 Structure of the lipid adjuvants with different types of linkers: (a) ether (DOTMA), (b) ester (DOTAP, pam2Cys, 306O$_{i10}$, SAL-12, DLin-MC3-DMA, SM-102, and ALC-0315), (c) amide (MIC-1, MIC-2, C1, and 3M-502), (d) dihydroimidazole (A2-Iso5-2CD18), and (e) alkyl (DDA).

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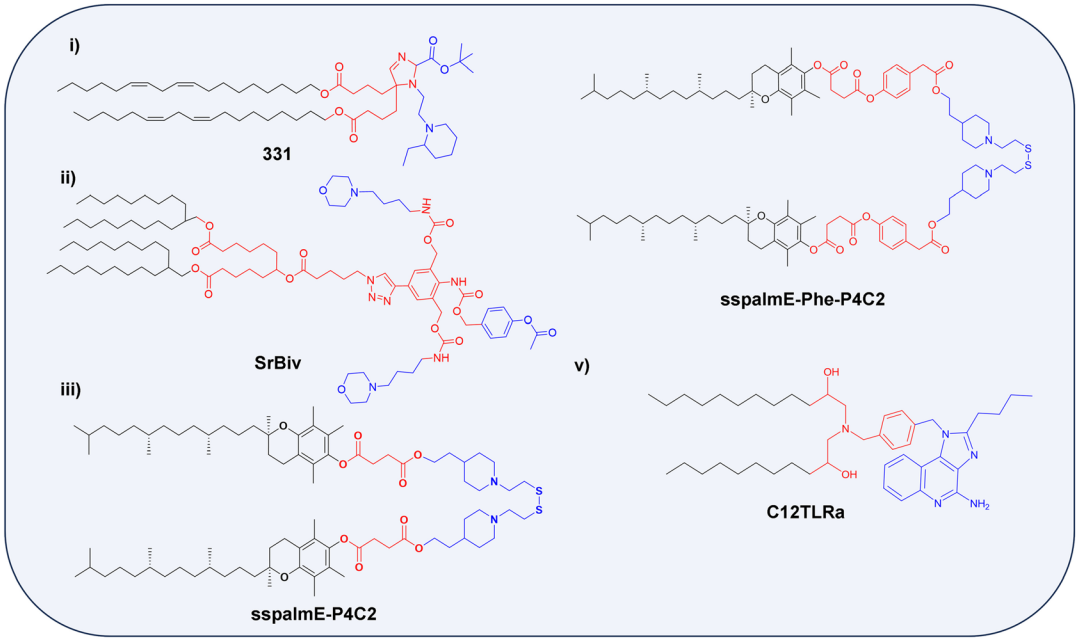

Fig. 6 Chemical structures of lipid adjuvants, highlighting their diverse linker chemistries such as (i) esters and imidazole (331), (ii) triazole, esters and carbamates (SrBiv), (iii) esters, phenyl ester and S–S bond (sspalmE-P4C2, sspalmE-phe-P4C2), and (iv) *para*-disubstituted benzene and tertiary amines (C12TLRa).

polar head and acyl chains have been explored. Optimizing linker chemistry is essential for developing next-generation lipid adjuvant vaccines with strong and lasting immune responses. The diversity and impact of these linkers are summarized in Table 1.

3.3.1. Ether. The incorporation of ether group linkers, exemplified by DOTMA, into lipid formulations enhances both the stability and efficacy of mRNA vaccine delivery systems. Ether linkages confer increased chemical stability and resistance to hydrolysis, thereby prolonging the functional lifetime of the lipid nanoparticles and improving the overall performance of mRNA vaccines.[8] The adjuvant efficacy and immunopotentiating properties of DOTMA are principally attributed to its unique molecular architecture, comprising two long-chain oleyl moieties and a quaternary ammonium head group interconnected *via* ether linkages. The incorporation of ether bonds imparts enhanced chemical and hydrolytic stability, superior transfection efficiency, reduced intrinsic immunogenicity, and improved cellular uptake. Additionally, the structural versatility conferred by ether linkages facilitates advanced formulation design. Collectively, these attributes position DOTMA as a highly effective component in contemporary gene delivery platforms and next-generation vaccine technologies.[17,33] Tahtinen *et al.* showed that LNPs containing DOTMA and DOPE act as potent adjuvants by significantly enhancing antigen-specific antibody production and robust Th1-type cellular immunity, including increased IFN-γ-producing T cells and germinal center-B cell responses. This immunostimulatory effect is mediated through the elevation of mitochondrial reactive oxygen species (ROS) and activation of the NOD-like protein receptor 3 (NLRP3) inflammasome, highlighting the advanced adjuvant properties of these LNPs.[36] DOTMA-containing LNPs robustly activate Th1 cells, leading to the production of antigen-specific antibodies through the induction of IFN-γ secretion. These lipids induce the secretion of IFN-γ and are found to be 60 times more potent than CpG-ODNs (a prime adjuvant) at generating Th1 cells. Additionally, cross-protection against influenza is provided by them, with IFN-γ being a crucial component of this resistance.[51]

3.3.2. Ester. Ester bonds, commonly formed through the condensation of carboxylic acids and alcohols, are a prevalent structural feature in lipid-based adjuvants. To overcome the limitations associated with the toxicity and reduced biodegradability of ether-linked lipids, Stamatatos *et al.* synthesized the cationic lipid DOTAP in 1988. DOTAP incorporates ester linkages, thereby enhancing its biocompatibility and facilitating its application as a safer and more effective adjuvant in lipid-based delivery systems.[52] DOTAP is a cationic lipid adjuvant composed of two oleyl hydrophobic chains and a quaternary ammonium head group, linked *via* two ester bonds, and exists as *R* and *S* enantiomers. When co-administered with a potent adjuvant and a tumor-specific antigen, DOTAP enhances dendritic cell activation and promotes robust T-cell responses, a process most effectively mediated by the (*R*)-enantiomer, while the (*S*)-enantiomer exhibits limited immunostimulatory activity. This enantiospecificity highlights the critical role of stereochemistry in DOTAP's adjuvant function and its capacity to potentiate antigen-specific cellular immunity in cancer vaccine formulations.[53] Shirai *et al.* demonstrated that DOTAP,

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Table 1 Overview of recently developed lipid adjuvants, detailing their structure, linker moieties, and associated immunological functions

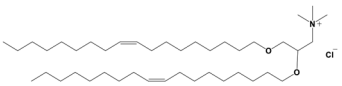
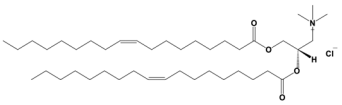
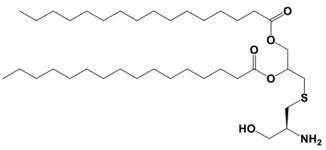
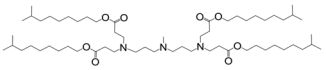
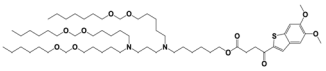
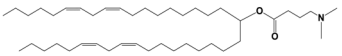
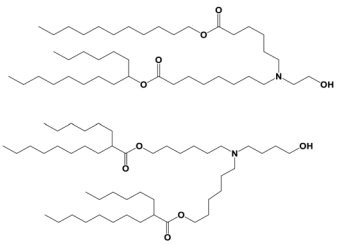
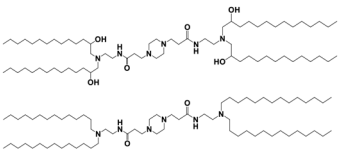
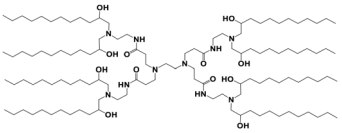
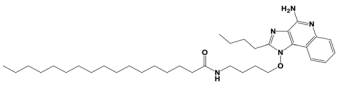
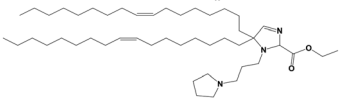
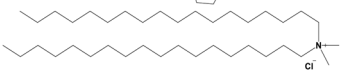
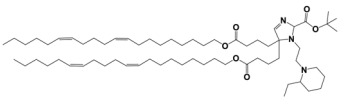
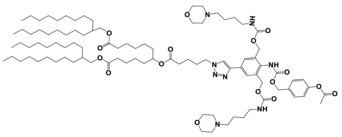
Adjuvant name	Linker	Function	Ref.
	Ether	• Increasing the amount of ROS in mitochondria is a significant mechanism for activating the NLRP3 inflammasome and promoting innate immunity	36
		• Encourages Th1 lymphocytes to produce antibodies against the specific antigen by releasing the cytokine IFN-γ	51
	Ester	• Activating the CD8 T cells and CD4 T cells by secreting cytokines like IFN-γ, and IL-2	56
		• An immunotherapeutic substance with properties associated with antigens and stimulates T and dendritic cell responses	57
		• Promotes humoral and cellular immunity in vaccinations, suggesting a possibility for creating secure effective, and successful vaccines against SARS-CoV-2 and influenza	58
	Ester	• A TLR2/6 agonist induces the cytokines like IL-12 and IL-17	59
	Ester	• Binding to TLR4 receptors and CD1d	60
	Ester	• By activating the STING pathway, producing the IFN-β cytokines, and inducing the antibodies against the SARS-CoV-2	61
	Ester	• Responsible for Th2 cell immune responses by triggering the IgG antibodies	62
	Ester	• Activating the TLR4 signaling through MyD88 and TRF adaptors, thereby promoting LNP-associated immune responses	63
	Amide	• Increase in RBD-specific IgG and neutralizing antibody levels and strong Th1-skewed T cell responses	66
	Amide	• C1 may increase immune responses by activating DCs and cytokines such as IL-6 and IL-12P70 by acting as a ligand for TLR4	67
	Amide	• Activation of B cells through TLR7	68
	Dihydroimidazole	• The activation of STING signalling induction, and promote the release of CXCL10 and IFNs	70
	Alkyl	• Responsible for Th1/Th17 immune responses through a CAF®01 against HIV and tuberculosis	73
	Esters and imidazole	• Larger antibody titers and more robust T-cell activation	75
	Triazole, esters, and carbamates	• Enhances the immune responses by increasing the ROS levels, attracting CD8+ T cells, and facilitating innate cell infiltration	78

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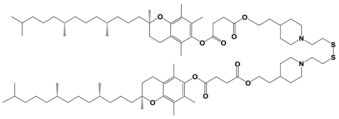
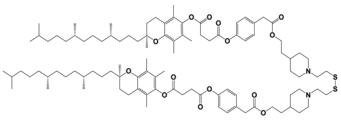
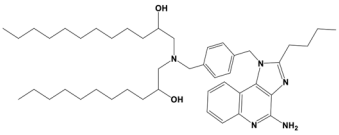
Table 1 *(Contd.)*

Adjuvant name	Linker	Function	Ref.
	Esters and S–S bond	• Induces cDC2 dendritic cells to function and activates cytotoxic T lymphocytes for immunological responses	80
	Esters, phenyl ester, and S–S bond	• Ready-to-use formulations that enhance antigen presentation to stimulate T cells and increase the strength of immune responses against cancer	28
	*para*-Disubstituted benzene and tertiary amines	• Activation of the TLR pathway of mRNA delivery with TLR7/8 agonist enhancing innate immunity	84

when used as an adjuvant in influenza split vaccine formulations in mice, effectively enhanced immune responses and conferred protection against influenza without inducing significant inflammatory reactions. This highlights DOTAP's capacity to potentiate vaccine efficacy while maintaining a favorable safety profile.[54] Haseda's group demonstrated that microfluidic-prepared DOTAP nanoparticles, owing to their small particle size, exhibit superior adjuvant properties compared to conventional liposomal DOTAP.[55] Henson *et al.* have shown that vaccination with (*R*)-DOTAP LNPs induces robust production of IFN-γ and IL-2, promoting potent CD8 T cell responses and antiviral activity in mice. Additionally, (*R*)-DOTAP alone was shown to activate, proliferate, and drive the differentiation of CD4 T cells toward a Th1 phenotype, supporting its potential as a simplified and safe adjuvant platform for human vaccination.[56] Gandhapudi *et al.* established the remarkable immunotherapeutic efficacy of the enantiomerically pure cationic lipid (*R*)-DOTAP as an antigen-specific adjuvant platform. (*R*)-DOTAP robustly enhances both T cell and dendritic cell activation, resulting in quantitatively strong antigen-specific cell responses and memory formation. In preclinical tumor models, (*R*)-DOTAP-based formulations significantly inhibited tumor growth, an effect further potentiated when combined with anti-PD1 checkpoint blockade, demonstrating synergistic antitumor activity and prolonged survival. Clinical trials have confirmed the safety and immunogenicity of (*R*)-DOTAP in humans, supporting its potential as a versatile and effective component of next-generation immunotherapies.[57] Recent studies by the same group showed that (*R*)-DOTAP, an enantiomerically pure cationic lipid, acts as a potent adjuvant in human subunit vaccines, inducing strong humoral and cellular immune responses. (*R*)-DOTAP significantly enhances antigen-specific antibody and T cell responses against influenza and SARS-CoV-2, highlighting its promise as a next-generation adjuvant for safe and effective advanced vaccine platforms.[58] Gu *et al.* developed Pam2Cys, a lipid adjuvant featuring a cystine-based head group, to enhance the immunogenicity of mRNA vaccines. As a toll-like receptor 2/6 (TLR2/6) agonist, Pam2Cys modulates immune responses within draining lymph nodes by inducing the production of cytokines such as IL-12 and IL-17. The demonstrated efficacy and immunostimulatory reliability of Pam2Cys in murine models suggest its potential to improve the clinical performance of next-generation mRNA vaccines.[59] Chaudhary *et al.* designed a series of ionizable lipids with amine head groups, among which the 306- and 313-head group-based variants demonstrated superior adjuvant properties. These lipids exert their immunostimulatory effects *via* direct interactions with toll-like receptor 4 (TLR4) and CD1d, resulting in the induction of Th1-skewed immune responses. This is evidenced by a marked elevation in antigen-specific IgG titers and a concomitant reduction in anti-PEG IgM levels. The engagement of LNPs with TLR4 and CD1d promotes the secretion of proinflammatory cytokines, thereby enhancing the immunogenicity of the vaccine formulation. Furthermore, both SM-102 and $306O_{i10}$ were shown to induce comparable early cytokine responses and maintain high delivery efficacy upon repeated administration, highlighting their potential for sustained and effective vaccine delivery in clinical applications.[60] SAL12, an amino lipid with an acid-labile hydrophobic tail and a built-in STING agonist, enables efficient Fluc mRNA delivery to bone marrow derived dendritic cells (BMDCs). SAL12-LNPs activate the STING pathway, enhancing neutralizing antibody production and immune cell differentiation, thus improving vaccine efficacy and providing durable protection against viruses like SARS-CoV-2. This highlights SAL12-LNPs as a promising next-generation mRNA vaccine platform.[61] Recently, Yavuz *et al.* reported that subcutaneous administration of DLin-MC3-DMA ionizable lipid nanoparticles modulates Th2-type immune responses, underscoring the influence of both lipid composition and delivery route on the qualitative nature of antibody-mediated immunity. Their findings demonstrated that this approach elicited comparable IgG titers and avidity indices in murine immunization models, indicating that DLin-MC3-DMA LNPs can effectively induce robust humoral responses characterized by Th2 polarization.[62] Collectively,

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these studies demonstrate that (*R*)-DOTAP, which contains a biodegradable and low-toxicity ester linker, exhibits potent adjuvant activity by effectively activating both CD8+ and CD4+ T cell responses. This underscores its promise as a safe and efficacious component in advanced vaccine formulations, aligning with the broader evidence that ester-based lipid adjuvants—including DOTAP and similar lipids—consistently display strong immunostimulatory properties.

Ionizable lipids in SARS-CoV-2 mRNA vaccine. Korzun *et al.* examined the reactogenicity and adjuvant characteristics of three lipids in mice: ALC-0315, SM-102, and DLin-MC3-DMA. They observed that by activating the TLR4 receptor, these LNPs promote the generation of cytokines, activate the complement system, and attract innate immune cells.[63] This has been demonstrated by Song *et al.*, where ALC-0315 and MC3-LNPs were shown to amplify cellular immune responses in mice following vaccination. Owing to its superior transport efficiency, stability, and immunostimulatory potential, ALC-0315 was found to be a more effective adjuvant than MC3 for eliciting both humoral and cellular immune responses.[64]

3.3.3. Amide. Amide linker-based cationic lipid adjuvants have been developed as a consequence of the presence of amide bonds in peptides and proteins. Amide linkages are formed *via* a condensation reaction between carboxylic acid and primary amine functionalities, resulting in the elimination of water and the formation of a chemically stable and biodegradable amide group.[65] In the innovative development of SARS-CoV-2 mRNA vaccines, Chen *et al.* synthesized six novel lipid adjuvants, among which MIC-1 and MIC-2 demonstrated superior performance relative to the SM-102 lipid. Incorporation of these adjuvants into the 4N4T-DS mRNA vaccine platform resulted in the induction of robust humoral immunity, as evidenced by markedly elevated titers of the receptor-binding domain (RBD)-specific IgG and neutralizing antibodies, alongside a pronounced Th1-skewed cellular immune response. These enhanced immunogenic outcomes have been attributed to the unique structural features of the lipid adjuvants, specifically the incorporation of an amide linker and a piperazine head group. Collectively, these molecular innovations have been shown to markedly improve both the efficacy and safety profiles of the mRNA vaccine.[66] The C1-mRNA nanovaccine represents a significant advancement in cancer immunotherapy, exhibiting potent antitumor efficacy with minimal observed toxicity. This innovative lipid adjuvant architecture integrates high-efficiency mRNA encapsulation with intrinsic self-adjuvant properties, enabling dual functionality: targeted delivery of tumor antigen-encoding mRNA to DCs *via* TLR4 signaling and activation of innate immunity through cytokine IL-2 and IFN-γ production.[67] Zimmermann *et al.* recently demonstrated that DDA/TDB/3M-052 liposomal formulations constitute a novel adjuvant system capable of eliciting type I immune responses in B cells, independent of the classical Th1, Th2, or Th17 pathways. The inclusion of 3M-052, a TLR7 agonist, within the lipid nanoparticles enables direct activation of B cells, resulting in rapid induction of IgG2c antibody responses. Notably, this B cell activation and class-switch recombination occur independently of T cell help, which is typically required for immunoglobulin class switching, thereby underscoring the unique immunomodulatory potential of this adjuvant platform.[68]

3.3.4. Dihydroimidazole. The incorporation of imidazole linkers into lipid formulations significantly augments the efficacy of gene delivery systems, particularly in the context of mRNA vaccines and other nucleic acid therapeutics. Their stability-enhancing properties, ability to facilitate membrane interactions, and pH sensitivity make them valuable components in modern pharmacological applications.[69] A2-Iso5-2DC18 (A2) is an advanced ionizable lipid-like material designed for mRNA vaccine delivery, characterized by a cyclic amine head group, a dihydroimidazole linker, and an unsaturated hydrophobic tail. This structural configuration enables efficient mRNA encapsulation and delivery while also imparting immunostimulatory properties. The A2 lipid adjuvant enhances immune responses by activating the STING signaling pathway, leading to the induction of type I interferons, pro-inflammatory cytokines, and C–X–C motif chemokines, thereby promoting robust antigen-specific cytotoxic T lymphocyte responses and potent antitumor immunity.[70]

3.3.5. Alkyl group. DDA is a cationic lipid adjuvant composed of two saturated octadecyl (C_{18}) alkyl chains and a positively charged quaternary ammonium head group linked *via* an alkyl moiety. Gall first recognized DDA as an effective lipid in 1966. DDA, a quaternary ammonium lipid, is a potent immune adjuvant especially for mRNA vaccines against rabies virus43[71] as it can conjugate with mRNA and produce innate immunity and Th1 cell responses. Previous studies have established that the DDA/TDB-based CAF®01 lipid formulation constitutes a highly promising adjuvant system, capable of eliciting robust immune responses while maintaining a favorable safety profile. Its ability to directly activate B lymphocytes and improve antigen delivery makes it a valuable component in modern vaccine strategies, particularly in developing vaccines for challenging pathogens like HIV and tuberculosis.[72] Wørzner *et al.* demonstrated that enhanced antigen adsorption to the CAF®01 adjuvant is positively correlated with lysozyme-specific Th1 and Th17 responses while exhibiting an inverse correlation with antibody titers in murine models. These results emphasize the importance of optimizing antigen–adjuvant interactions to significantly impact humoral and cell-mediated immune responses, highlighting CAF®01's potential as a versatile adjuvant for future-generation vaccines.[73]

3.3.6. Multi-functional linkers

Esters and imidazole. In their study, Zhao *et al.* identified imidazole-containing lipids as particularly efficacious for T-cell transfection. They demonstrated that transfection efficiency is modulated by the spatial arrangement, specifically the carbon chain length separating the imidazole and amine groups, as well as by the structural design of the heterocyclic rings. Additionally, the presence of heteroatoms within these lipid structures was found to facilitate efficient mRNA delivery.[74] Li *et al.* demonstrated that ionizable lipids having cyclic amine head groups function as potent adjuvants or immunos-

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timulatory ligands in mRNA vaccine formulations. Among these, Lipid 331—a biodegradable, immunostimulatory lipid—exhibited superior adjuvant activity compared to ALC-0315, resulting in markedly enhanced T-cell activation and significantly elevated antibody titers when formulated as LNPs. This underscores the critical role of rational head group design in optimizing both cellular and humoral immune responses in next-generation mRNA vaccines.[75]

Triazole, esters, and carbamates. The triazole heterocyclic aromatic ring is employed as a linker in cationic/ionic lipid adjuvants due to its exceptional chemical stability across acidic, basic, oxidative, and reductive environments.[76] Furthermore, the incorporation of biodegradable ester bonds imparts stability to the system at neutral pH while enabling controlled hydrolytic degradation. Conversely, carbamate bonds within the same framework exhibit susceptibility to acid-catalyzed hydrolysis under specific conditions, affording pH-responsive degradation profiles.[77] A bivalent ionizable lipid which is stimuli-responsive was synthesized by Dong *et al.*, by incorporating three distinct linker moieties, functioning as an adjuvant to enhance mRNA translation and elicit robust antitumor immune responses. This lipid formulation augments cancer immunotherapy by elevating reactive oxygen species (ROS) levels and modulating tumor progression. Combined with vaccination and anti-PD-1 checkpoint blockade, the system exerts potent systemic antitumor effects by restoring antigen-specific $CD8^+$ T cell activity, thereby underscoring the adjuvant potential of this lipid platform in advanced cancer immunotherapeutic strategies.[78]

Esters, phenyl ester, and S–S bond. Disulfide bonds have attracted considerable attention in the design of cationic lipids due to their redox-responsive nature; these bonds are formed *via* the oxidation of sulfhydryl groups and are readily cleaved by intracellular glutathione, thereby enabling the efficient release of DNA from lipoplex complexes into the cytoplasm.[79] Oyama *et al.* recently synthesized an ionizable lipid adjuvant, sspalmE-P4C2, featuring an amine head group, pH-responsive disulfide-cleavable linker moieties, and a vitamin E-derived hydrophobic domain. This advanced lipid formulation enhances the function of conventional dendritic cell subtype 2 (cDC2), facilitating robust activation of cytotoxic T lymphocytes (CTLs). When utilized in mRNA vaccine platforms encoding ovalbumin (OVA), sspalmE-P4C2 nanoparticles effectively promote antigen expression within cDC2 cells, leading to potent CTL-mediated antitumor responses in the E.G7-OVA tumor model. By stimulating innate immune pathways and enhancing cytoplasmic antigen presentation, this ionizable lipid adjuvant demonstrates significant potential as a next-generation immunotherapeutic platform for cancer immunotherapy.[80] Leveraging the self-degradable nature of phenyl esters and the immunostimulatory properties of vitamin E scaffolds, Anindita *et al.* developed the ionizable lipid adjuvant ssPalmE-Phe-P4C2. This formulation enhances mRNA encapsulation efficiency, stability, and shelf life, particularly when combined with freeze-drying techniques. The resulting vaccine platform significantly improves antigen presentation and immune activation, thereby potentiating antitumor immune responses. This innovative lipid adjuvant strategy demonstrates substantial promise for increasing the efficacy of carcinoma vaccines and highlights the potential of advanced lipid engineering in cancer immunotherapy.[28]

para-Disubstituted benzene and tertiary amines. Chang's group and others have demonstrated that the incorporation of aromatic moieties into lipid structures significantly enhances transfection efficiency, likely through π–π stacking and interactive interactions with nucleic acid base pairs, thereby improving DNA binding and complex stability.[81] Secondary and tertiary amine functionalities—prevalent in polymeric and lipid-based vectors—are critical for establishing electrostatic interactions with DNA, facilitating complex dissociation, and promoting efficient cytosolic release of cargo.[82,83] Recognizing the functional advantages conferred by linker design, Han *et al.* engineered a C12-TLRa ionizable lipidoid capable of efficiently encapsulating and delivering SARS-CoV-2 mRNA to target cells, with a particular affinity for APCs. This adjuvant lipidoid augments the immunostimulatory properties of lipid nanoparticles by activating toll-like receptor (TLR) signaling pathways, thereby potentiating both innate and adaptive immune responses. This study provides a foundational basis for utilizing adjuvant lipidoids in lipid nanoparticle formulations to improve the quality and magnitude of adaptive immune responses for mRNA vaccines. By enhancing both innate and adaptive immunity, this approach holds promise for developing more effective vaccines against SARS-CoV-2 and potentially other infectious diseases.[84]

4. Current status of lipid adjuvants in LNP-based mRNA vaccine and limitations

The field of lipid-adjuvanted mRNA vaccine technology is undergoing rapid expansion, with numerous clinical trials currently evaluating its efficacy across a spectrum of immunotherapeutic indications. The advancement of mRNA vaccine platforms has been largely facilitated by the favorable safety profiles, robust immunogenicity, and precise targetability conferred by lipid-based delivery systems.[85] Integration of liposomal technology with mRNA vaccines has yielded promising clinical outcomes in humans for a variety of diseases, including malaria, influenza, tuberculosis, HIV, and shingles.[86] The global spotlight on mRNA vaccines intensified following the expedited development and successful clinical deployment of LNP-based vaccines against SARS-CoV-2, underscoring the platform's potential for rapid response to emerging infectious threats.[87] Ongoing research continues to broaden the therapeutic landscape of mRNA vaccines, with active investigations into their application in cancer immunotherapy, restoration of tumor suppressor gene function, and genome editing. These advances highlight the versatility and transformative potential of mRNA-LNP technology for a wide array of gene-based thera-

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peutic interventions. In addition, the *in situ* generation of chimeric antigen receptor (CAR) cells through CAR mRNA delivery has emerged as a highly promising strategy in cancer immunotherapy.[88] LNPs have demonstrated efficacy in delivering mRNA encoding tumor-associated antigens (TAAs), co-stimulatory molecules, cytokines, tumor suppressor genes, Cas9 endonuclease, and CAR and T cell receptors (TCRs). This approach enables potent and targeted modulation of the immune response, facilitating the development of advanced mRNA-based cancer therapeutics.[89]

The delivery efficiency of mRNA can be significantly improved through the rational design of lipid molecules, specifically by optimizing the chemical structure of polar head groups and hydrophobic tails. Such modifications facilitate enhanced cellular uptake and promote efficient endosomal escape of lipid nanoparticle-encapsulated mRNA formulations.[90] Notably, LNPs have demonstrated superior adjuvant activity compared to AddaVax, a commonly utilized adjuvant. Although the LNP-based COVID-19 mRNA vaccines do not include traditional adjuvants, the robust cellular and humoral immune responses elicited against SARS-CoV-2 are likely attributable, at least in part, to the intrinsic immunostimulatory properties of the LNP formulation itself.[91] A recent study by the Korzun group demonstrated that mRNA vaccine nanocarriers such as ALC-0315 and SM-102 possess intrinsic adjuvant properties by activating TLR4 in murine models.[63] Understanding the relationship between lipid structure and its functional properties, including efficacy and adjuvant activity, remains a significant challenge. Although considerable research efforts have been directed toward elucidating the underlying mechanisms, the precise correlations are not yet fully established. Despite ongoing attempts to associate lipid structural features with their immunostimulatory and adjuvant properties, further insights are essential for the rational design of lipid adjuvants tailored to specific disease indications. Investigations into the structural modifications of lipid adjuvants are critical for advancing our understanding of their immunomodulatory behavior in mRNA vaccine formulations. In this context, we have discussed the role of individual lipid components within these formulations and their contributions to eliciting immune responses. Notably, ionizable and cationic lipids have emerged as key determinants of adjuvant activity in mRNA vaccines. This review primarily focuses on how structural variations—particularly in the hydrophobic tails, polar head groups, and linker regions—of contemporary ionizable and cationic lipid adjuvants influence their adjuvant properties.

5. Advancement of clinical frontiers in lipid adjuvants

The advancement of mRNA vaccine technology has been propelled by its demonstrated efficacy across a range of diseases, and ongoing research into these innovative formulations is poised to yield next-generation vaccines with enhanced immunogenicity and broader therapeutic applicability. Lipid adjuvants, particularly those integrated within LNP delivery systems, have achieved substantial clinical milestones in recent years, notably in the domains of vaccine development and RNA-based therapeutics. Presently, the majority of clinical investigations focus on LNP-mediated delivery platforms for mRNA vaccines, underscoring their pivotal role in the evolution of precision medicine and immunotherapy.[92] Patisiran (ONPATTRO®) is the first FDA-approved RNA interference (RNAi) for the treatment of polyneuropathy associated with hereditary transthyretin-mediated (hATTR) amyloidosis in adults. This double-stranded small interfering RNA (siRNA) is formulated within an LNP delivery system, which incorporates the ionizable lipid D-Lin-MC3-DMA as an adjuvant, along with helper lipids such as DSPC, cholesterol, and DMG-PEG-2000.[93] An LNP-based rabies mRNA vaccine encoding the rabies virus glycoprotein (RABV-G) has been evaluated in a Phase I clinical trial in humans. The LNP formulation consists of a cationic lipid, DSPC, cholesterol, and PEG lipid, encapsulating mRNA for efficient antigen expression and immunogenicity.[94] Monophosphoryl lipid A (MPL) is a clinically licensed agonist of TLR4 that is utilized as an adjuvant in several human vaccines. MPL enhances immune responses by activating TLR4-mediated signaling pathways.[95] The most significant advancement in clinical translation has been the deployment of LNP platforms, exemplified by the COVID-19 mRNA vaccines—Moderna's mRNA-1273 and Pfizer-BioNTech's BNT162b2—both of which utilize LNP-encapsulated mRNA and have demonstrated robust safety and efficacy profiles in large-scale clinical trials.[92] Ongoing clinical investigations are evaluating a diverse array of mRNA vaccines incorporating lipid-based adjuvants, with a primary focus on their safety, immunogenicity, and therapeutic efficacy. These studies are extending to vaccines targeting influenza and other respiratory pathogens, leveraging the proven clinical success and technological foundation established by COVID-19 mRNA vaccine development.[96,97] Widely studied cationic lipid adjuvants such as DOTMA, DOTAP, and DDA have demonstrated superior performance in clinical applications. These lipid-based formulations have leveraged the intrinsic immunostimulatory properties of their components to facilitate rapid advancement from preclinical evaluation to global clinical deployment, yielding robust immunogenicity and favorable safety profiles.

Recent advances in lipid-based adjuvants have demonstrated enhanced immune activation in murine models, primarily through TLR engagement and downstream signaling pathways. While these adjuvants exhibit superior immunostimulatory efficacy in preclinical studies, ongoing human clinical trials are assessing their safety, immunogenicity, and therapeutic effectiveness.[98] Recent advances in immunoengineering have facilitated the development of specialized adjuvant lipidoids—synthetic lipid-based molecules engineered to serve dual functions as both delivery vectors and immunostimulatory agents. The incorporation of TLR agonist-derived lipidoids into LNP formulations has been shown to potentiate both innate and adaptive immune responses in preclinical and translationally relevant vaccine models. Experimental data indicate that the integration

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of adjuvant lipidoids into clinically relevant mRNA vaccine platforms markedly augments immunogenicity, enhancing antigen presentation and effector cell activation. The use of lipid adjuvants within LNPs has accelerated the clinical translation of mRNA vaccines and RNA therapeutics, with ongoing innovations in lipidoid design and formulation poised to enable broader, more potent, and more durable immunotherapeutic applications in future clinical settings.

6. Summary and future directions

Structural modification of lipid adjuvants exerts a profound influence on their biological activity, including pharmacokinetic and pharmacodynamic profiles, immunomodulatory capacity, and overall therapeutic efficacy.[99] Advancements in synthetic methodologies and elucidation of SARs are pivotal for the rational optimization of lipid adjuvants in mRNA vaccine platforms. The primary objective of these engineered lipid adjuvants is to augment delivery efficiency and immunogenicity by enabling the design of next-generation LNPs with tailored physicochemical and immunostimulatory properties.[100] These advancements are directed toward enhancing both innate and adaptive immune responses while preserving favorable safety profiles. Emerging evidence indicates that structural modifications of lipid adjuvants exert a profound impact on immunogenicity and delivery efficiency in next-generation formulations. Specifically, alterations in lipid architecture—including variations in head group chemistry, hydrophobic tail composition, and linker functionality—have been shown to modulate antigen presentation, immune cell activation, and overall vaccine efficacy.[101] Head groups containing tertiary amines, amino acids, and heterocycles exhibit greater adjuvant activity than quaternary amines, likely due to improved ionizability, membrane interaction, and endosomal escape, thereby enhancing the potency and efficacy of lipid-based adjuvants in mRNA vaccines.[60]

The adjuvant properties of quaternary amines have been primarily characterized in compounds such as DOTMA, DOTAP, and DDA. Further investigation is required to confirm these observations and understand the underlying mechanisms. Hydrophobic moieties, including oleic acid, long-chain alkyl structures, and tocopherol derivatives, have demonstrated robust immunostimulatory effects, serving as effective hydrophobic domains within lipid adjuvant architectures. Additionally, the incorporation of degradable linkers—such as esters and amides—has been shown to enhance adjuvant activity by promoting activation of TLRs, STING pathways, and Th1-mediated immune responses, which are critical for both innate and adaptive immunity. Conversely, non-degradable linkers like ethers generally exhibit limited adjuvant potential; however, the ether-linked quaternary amine DOTMA represents a notable exception, having demonstrated exceptional adjuvant efficacy. These findings indicate that, while degradable linkers generally potentiate immune responses, select ether-linked compounds can also exhibit substantial adjuvant efficacy. Notably, the alkyl linker-based lipid adjuvant DDA demonstrates highly favorable immunostimulatory properties; consequently, the DDA-based CAF01 platform has emerged as a promising adjuvant system with demonstrated clinical utility in HIV and tuberculosis vaccine trials. Heterocyclic ring-containing linkers, such as imidazole and triazole derivatives, confer excellent adjuvant characteristics, attributed to their low cytotoxicity and enhanced facilitation of endosomal escape. Furthermore, multifunctional linker-based lipids, particularly those incorporating disulfide and other stimuli-responsive motifs, have shown pronounced adjuvant activity. Disulfide-cleavable linkers, especially when combined with degradable esters and phenyl esters, have been shown to robustly activate immune responses, underscoring their potential as superior adjuvant components in advanced vaccine formulations. TLR activation has also been observed with lipid adjuvants featuring tertiary amine and *para*-disubstituted benzene linkers in combination with heterocyclic head groups. Overall, multifunctionalized linker-based lipids—incorporating either identical or diverse linker chemistries—tend to exhibit superior adjuvant properties compared to their single-linker counterparts, with certain structures demonstrating exceptional efficacy. Further investigation is warranted to elucidate the mechanisms underlying the immunomodulatory behavior of multifunctionalized lipid adjuvants. This review highlights the critical impact of structural variations in lipid adjuvants on immune response profiles, emphasizing the need for continued research to advance mechanistic understanding in this field.

Author contributions

SC was responsible for data collection, management, maintenance, design, and organization. PH critically revised and edited the manuscript. SM oversaw project progression and contributed to the initial manuscript draft. SP supervised overall project progress, participated in manuscript revision and editing, and secured financial support for the study.

Conflicts of interest

There are no conflicts to declare.

Data availability

No primary research results, software or code have been included and no new data were generated or analysed as part of this review.

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