Khalifa, A. Z., Perrie, Y., & Shahiwala, A. (2025). Subunit antigen delivery: emulsion and liposomal adjuvants for next-generation vaccines. Expert Opinion on Drug Delivery, 1-15. Advance online publication. https://doi.org/10.1080/17425247.2025.2474088.

Subunit antigen delivery: emulsion and liposomal adjuvants for next-generation vaccines

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Abstract

Introduction: Developing new vaccines to combat emerging infectious diseases has gained more significance after the COVID-19 pandemic. Vaccination is the most cost-effective method for preventing infectious diseases, and subunit antigens are a safer alternative to traditional live, attenuated, and inactivated vaccines.

Areas covered: Challenges in delivering subunit antigens and the status of different vaccine adjuvants. Recent research developments involving emulsion and liposomal adjuvants and their compositions and properties affecting their adjuvancy.

Expert opinion: Lipid-based adjuvants, e.g., emulsions and liposomes, represent a paradigm shift in vaccine technology by enabling robust humoral and cellular immune responses with lower antigen doses, a property that is particularly critical during pandemics or in resource-limited settings. These adjuvants can optimize vaccine administration strategies by potentially reducing the frequency of booster doses, thereby improving patient compliance and lowering healthcare costs. While emulsions excel in dose-sparing and broadening immune responses, liposomes offer customization and precision in antigen delivery. However, the broader clinical application of these technologies is not without challenges. Stability issues, e.g., the susceptibility of emulsion-based adjuvants to freezing and their reliance on cold-chain logistics, pose significant barriers to their use in remote/underserved regions. Future developments will likely focus on improving manufacturing scalability and cost-effectiveness.

Keywords: Vaccines; subunit antigens; adjuvants; lipid-based particles; emulsion; liposomes

Article Highlights:

- While subunit vaccines offer an improved safety profile over live or inactivated vaccines, their reduced immunogenicity necessitates potent adjuvants for effective immune stimulation.
- Adjuvants enhance antigen presentation, promote antigen-presenting cell maturation, and trigger cytokine production and chemokines, while delivery systems are designed to stabilize antigens, control their release, and target them to specific immune cells or tissues, thereby improving the efficiency and specificity of the immune response.
- Lipid-based adjuvants, including emulsion and liposomal formulations, are recognized for their biodegradability, biocompatibility, and the capacity to be precisely tailored.
- Emulsion adjuvants like MF59 and AS03 boost immune responses by creating antigen depots that extend exposure and by triggering local inflammation to recruit and activate antigen-presenting cells.
- The adjuvant efficacy of liposomes is critically influenced by factors such as vesicle size, surface charge, lipid composition, and the mode of antigen incorporation (encapsulation vs. surface adsorption).
- Emerging manufacturing techniques such as microfluidic production and the development of personalized adjuvant systems hold promise for next-generation vaccines.

1. Introduction

Vaccination remains the most cost-effective method for preventing infectious diseases and has been a cornerstone of public health for decades (1). While traditional vaccines, such as live attenuated and inactivated vaccines, have demonstrated efficacy, concerns regarding safety and the potential for reversion to virulence have spurred the development of alternative vaccine platforms (2,3). Later, subunit vaccines emerged in which only purified antigens were employed in developing the vaccine instead of the whole micro-organism. Although subunit vaccines are safer than traditional vaccines, they are less immunogenic (4,5).

Adjuvants and delivery systems are critical components of modern vaccine formulations. Adjuvants function by stimulating the immune system to elicit robust and durable immunity. They achieve this through various mechanisms, including enhancing antigen presentation, promoting the maturation of antigen-presenting cells (APCs), and triggering the production of cytokines and chemokines (6,7). Delivery systems, on the other hand, are designed to stabilize antigens, control their release, and target them to specific immune cells or tissues, thereby improving the efficiency and specificity of the immune response (8,9). For instance, aluminum salts, one of the earliest adjuvants, work by forming antigen depots that sustain antigen release over time (10), while newer systems, such as emulsions and liposomes, provide additional functionalities like immune modulation and targeted delivery (11). Table 1 shows FDA-approved subunit vaccines used with the employed adjuvants and delivery systems.

Table 1: FDA-approved subunit vaccines (12)**.**

Adjuvant	Vaccine	Subunit Antigen	Route of Administration	Year of approval
Alum	Haemophilus B Conjugate Vaccine (Meningococcal Protein Conjugate) (PedvaxHIB)	Capsular polysaccharide of *Haemophilus influenzae* type b, covalently bound to an outer membrane protein complex of Neisseria meningitidis serogroup B	IM	1990

Alum	Hepatitis B Vaccines (Recombivax HB, PREHEVBRIO, Engerix-B)	Recombinant hepatitis B virus surface antigen (HBsAg)	IM SC	 1986 2021 1989
Alum	Human Papillomavirus Vaccine (Gardasil)	Purified virus-like particles (VLPs) of recombinant major capsid (L1) protein of HPV.	IM	2014
Alum	Meningococcal Group B Vaccine (TRUMENBA)	Recombinant lipidated factor H binding protein (fHbp) variants from *Neisseria meningitidis* serogroup B	IM	2014
Alum	Meningococcal Group B Vaccine (BEXSERO)	Recombinant proteins of Neisserial adhesin A (NadA), Neisserial Heparin Binding Antigen (NHBA), factor H binding protein (fHbp) and Outer Membrane Vesicles	IM	2015
MF59, squalene-based oil-in-water emulsion	Influenza A (H5N1) Monovalent Vaccine (AUDENZ)	Surface antigen, hemagglutinin (HA) of influenza virus	IM	2020
MF59, squalene-based oil-in-water emulsion	Influenza Vaccine (Fluad)	Purified hemagglutinin and neuraminidase surface antigens of 3-4 influenza strains	IM	2015

AS04, monophosphoryl lipid A (MPL) adsorbed onto aluminum	Human Papillomavirus Bivalent (Types 16 and 18) Vaccine (Cervarix*)	Virus-like particles (VLPs) of recombinant L1 protein, the major antigenic protein of the capsid of oncogenic HPV types 16 and 18	IM	2009
AS01B, monophosphoryl lipid A (MPL) and QS-21, Quillaja saponins combined in liposomes	Zoster Vaccine (SHINGRIX)	Recombinant varicella-zoster virus surface glycoprotein E (gE) antigen component	IM	2017
Matrix-M, saponin extracts in liposomes	COVID-19 vaccine (Novavax)	SARS-CoV-2 recombinant spike (rS) protein	IM	2022

(*) Has been discontinued from the market.

A vaccine adjuvant and a vaccine delivery system have often been used interchangeably in relation to vaccines, but it is important to distinguish between them and to differentiate their respective roles more clearly, especially when a vaccine adjuvant is delivered by a delivery system. The potency of these delivery systems can be significantly improved by the addition of a vaccine adjuvant, or immunostimulants. Also, therapeutic ratio of adjuvants can be increased by adding them to delivery systems to target their effects toward APCs and to minimize their effects on non-immune cells. Vaccine delivery systems, such as emulsions, microparticles, iscoms, liposomes, virosomes, and virus-like particles, are comparable in size to pathogens that the immune system

evolved to fight. Because of this, these particles are generally taken up efficiently by APCs and deliver the associated antigens to them (8).

Recent advances in vaccine technology have brought lipid-based adjuvants and delivery systems to the forefront. Emulsions, such as MF59 and AS03, are squalene-based oil-in-water formulations that enhance immune responses by creating an immunocompetent environment at the injection site (13). Similarly, liposomes are versatile vesicular carriers composed of phospholipid bilayers that can encapsulate hydrophilic and lipophilic antigens (14). Their tunable properties, such as size, surface charge, and lipid composition, allow for precise customization to elicit specific immune responses (14). These systems have demonstrated the ability to enhance both humoral and cellular immunity, addressing key challenges in vaccine development, particularly for subunit vaccines. Furthermore, these technologies offer opportunities for improving vaccine stability, reducing the need for cold-chain storage, and enabling rapid adaptation to emerging pathogens rate (15).

The integration of adjuvants and delivery systems into vaccine development represents a significant paradigm shift. By optimizing these components, it is possible to tailor vaccines to meet diverse immunological needs, from eliciting strong mucosal immunity to targeting specific populations such as the elderly or immunocompromised (16). This review provides an in-depth analysis of the distinct roles of adjuvants and delivery systems in enhancing the efficacy of subunit vaccines. It examines the challenges associated with subunit vaccine delivery, the mechanisms by which emulsions and liposomes enhance immune responses, and the potential of lipid-based technologies to address the limitations of traditional vaccine approaches. Additionally, recent advances and future directions in the development of these systems for various infectious diseases are discussed. The subsequent sections explore the mechanisms, compositions, and applications of these technologies in more detail.

2. Challenges in Delivering Subunit Antigens

Different types of antigens result in variable immune responses (3), and all antigen-related factors, including the dose, frequency of administration, and kinetics, could have an impact (17). Additionally, adjuvants are necessary to overcome the lower potency of subunit vaccines (2,18). Adjuvants are molecules or delivery systems that cause immune stimulation and enhance the immune response (19). This opens the new challenge of selecting an ideal adjuvant to produce an

optimum immune response with the required antigen. The adjuvant should achieve maximum immune activation with the least adverse reactions. It should be well-tolerated and biocompatible (20). The immunogenicity of the vaccine is influenced by the type, nature, and size of the adjuvant used (8,9). It could be an immune-potentiating compound, the delivery system (also immune-potentiating), or a combination (20).

Aluminum salt adjuvants are the most commonly used adjuvants in vaccines (10). These are known as 'alum' (21). However, this term correctly applies only to aluminum potassium phosphate (22). It was initially reported by Glenny et al. that precipitating the antigen onto insoluble aluminum potassium phosphate resulted in a better antibody response than that elicited by the antigen on its own, and this was believed to be due to the depot effect of the insoluble salt particles, which allow the gradual release of the antigen and thus the prolonged stimulation of the immune system (23). It was later found that these insoluble aluminum salts can also activate the immune cells (24).

Two primary forms of aluminum salt adjuvants are commonly used: aluminum hydroxide and aluminum phosphate adjuvants. The adsorption of antigens to preformed aluminum salt adjuvants is more reproducible and better standardized than the initial precipitation technique (25). The adsorption mechanism could be owed to electrostatic attraction when the antigen and adjuvant carry opposite charges or ligand exchange when antigens have terminal phosphate groups, as aluminum has a higher affinity to phosphates than hydroxyl (22).

Both forms of aluminum salt adjuvants have different properties, resulting in various immune responses (22). Many factors affect their efficiency as adjuvants; those include the rate and strength of their adsorption with the antigen, their particle size and uniformity, their dosage, and the characteristics of the antigen (26). However, in clinical studies, alum was found to be less potent when compared with other adjuvants like oil-in-water emulsions. It was shown to be a poor inducer of Th-1-associated immune responses (27). One of the limitations of aluminum salt adjuvants is their thermostability as they cannot be frozen or lyophilized, and in turn, the vaccines that comprise them (28,29). Also, alum has been considered acceptably safe for many years, but it still induces local reactogenicity (30), and aluminum is a known neural toxin (31). The adsorption of protein antigens onto different aluminum salts reduced the stability of the proteins significantly and irreversibly. The extent of destabilization varied between different proteins, which was accompanied by disruption of the protein structure upon interaction with the salt surfaces (32).

The adjuvant selection and design are critical since they control the delivery and presentation of antigens to APCs. It should allow the specific delivery of the immune potentiators to their target cells (20). Particulate delivery systems are favorable as their sizes are similar to natural pathogens (8) and thus will be identified by the natural immune system uptake and recognition mechanisms (16). In some cases, even if an effective antigen and adjuvant are used, the vaccine might still exhibit a poor outcome. A particular focus should be directed toward formulation to achieve an efficient, stable, and safe subunit vaccine (33). Here comes the challenge of designing and tackling different formulation approaches to develop a delivery system suitable for the intended route by which the vaccine will be administered (34).

It is essential to control the physicochemical characteristics of the resulting particulate delivery system. This includes its size and surface charge, which will influence its interaction and uptake by APCs and, therefore, its immune response (35). The immune response is also influenced by the mechanism by which the antigen is associated with the adjuvant (27). Furthermore, combining the antigen with the adjuvant could induce changes in the antigen surrounding, like pH, ionic strength, and temperature, which will cause conformational changes in the antigen and, in turn, a change in its stability and immunogenicity.

Polymer-based particulate adjuvants offer several advantages, including an improvement in the stability of antigens and modification of their release kinetics. They provide a controlled release of antigens to stimulate an immune response while providing a depot effect. Moreover, adjustment of their different properties could result in better antigen uptake, processing, and presentation (36,37). They can be made from different polymers such as chitosan, poly (lactic-co-glycolic acid) (PLGA), poly(lactic acid) (PLA), poly(glutamic acid) (PGA), and acrylic acid-based polymers. Biodegradable natural and synthetic polymers can control the release of antigens and enhance their immunogenicity. Their adjuvant efficiency is related to their solubility, molecular weight, degree of branching, and conformation (38).

Various methods could be used to prepare polymer-based particles, termed microparticles or nanoparticles, depending on whether their size is above or below 1000 nm. The compounds of interest are entrapped through dissolving, wrapping, or adsorption on their surface (39). It is essential to control the particles' size and surface properties as they will influence their adjuvant effect (40). Controlled antigen release from the particles depends on their size, polymer

composition, matrix porosity, and whether the antigen is entrapped within the particle's matrix or adsorbed to their surface (41). When the antigen is entrapped within the particle matrix, its release can occur either by diffusion through a tortuous, water-filled path in the polymer matrix or by polymer erosion (42). Therefore, the release rate will depend on the diffusion or polymer erosion and degradation rates. Still, when the antigen is attached to the particle surface, its release rate will depend on the interaction force governing its association (43).

Natural polymers involve different polysaccharides which originate from plants and microorganisms. Examples include dextran derivatives, lentinan, inulin, mannan, chitosan, and PGA (44). Synthetic polymers include polyphosphazenes, polyelectrolytes, polyanhydrides, poloxamers/pluronics, polymethacrylates, polyglycolic-co-lactides, polycaprolactones, and polyvinylpyrrolidone (38). These polymers are biocompatible, biodegradable, and non-toxic, making them better than aluminum salt adjuvants (45,46). Their small size range also allows their uptake and retainment by the lymphatic system, producing an immune response without the need for recurrent dosing (38). However, polymer-based adjuvants are often poorly immunogenic and the co-administration with molecular adjuvants is often used to improve and direct the immune response (47).

3. Lipid-Based Vaccine Delivery Systems

Lipid-based adjuvants include emulsions, liposomes, immune-stimulating complexes (ISCOMs), cubosomes, virosomes, and archaeosomes. These are considered attractive adjuvants since they are biodegradable, biocompatible, affordable, and can be easily customized for different vaccines by varying their composition (11). Their particulate nature makes them comparable in size to pathogens and, therefore, could be delivered to APCs and migrate to lymph nodes. Moreover, the slow degradation of those particles means slow clearance of the carried antigens (48). Entrapping the antigens can also protect them from possible enzymatic degradation (49). Specifically, emulsions and liposomes are the most widely studied and under clinical trials (Table 2).

Table 2: Emulsion and Liposome-Based Adjuvants in Clinical Trials (accessed on 28th November 2024).

ClinicalTrial. Gov Identifier	Antigen	Adjuvant	Disease/ Condition	Phase	Sponsored by
NCT03961438	Recombinant HIV-1 Envelope Protein ConM SOSIP.v7 gp140	Monophosphoryl lipid A (MPLA) liposomes	HIV-1-infection	1	Emma Reiss, Academisch Medisch Centrum - Universiteit van Amsterdam (AMC-UvA)
NCT03934541		HIV-1 gp41 MPER-656 liposome	HIV infection	1	National Institute of Allergy and Infectious Diseases (NIAID)
NCT00020462	Tumor-Derived Antigen and IL-2	Liposome	Lymphoma	1	National Cancer Institute (NCI)
NCT05208125	ChAdOx1.HTI and MVA.HTI with Recombinant HIV-1 Envelope Protein ConM SOSIP.v7 gp140	MPLA Liposomes	HIV infection	1	IrsiCaixa
NCT00197301	Influenza	Liposomes	Influenza	1,2	Hadassah Medical Organization
NCT01052142	Dendritic cells	Liposomes	Melanoma	1	Lipotek Pty Ltd
NCT00157209	Tecemotide (BLP25)	Liposomes	Carcinoma, Non-Small-Cell Lung Neoplasms	2b	Merck KGaA, Darmstadt, Germany
NCT01556789	Synthetic glycolipopeptide MUC1 antigen, M40Tn6, and novel synthetic TLR-4 agonist, PET Lipid A (ONT-10)	Liposomes	Solid Tumors	1	Cascadian Therapeutics Inc.

NCT01094548	Synthetic 25-amino acid lipopeptide derived from the tandem repeat region of MUC1 glycoprotein (Tecemotide)	Monophosphoryl lipid A Liposomes	Multiple Myeloma	2	Merck KGaA, Darmstadt, Germany
NCT00960115	Tecemotide	Monophosphoryl lipid A Liposomes	Multiple Myeloma	1,2	Merck KGaA, Darmstadt, Germany
NCT01978964	ONT-10	Liposomes	Solid Tumors	1b	Cascadian Therapeutics Inc.
NCT00000749	gp120 (CHO) BIOCINE	MF59 Emulsion	HIV Infections	1	National Institute of Allergy and Infectious Diseases (NIAID)
NCT00001019	HIV-I_{SF2} gp120 glycopeptides	MF59 emulsion alone or with MTP-PE/MF59 adjuvant	HIV Infections	1	National Institute of Allergy and Infectious Diseases (NIAID)
NCT01098786	Cell-derived A/H1N1 Influenza HA virus	Emulsion	Swine-Origin Influenza A H1N1 Virus		Novartis Vaccines
NCT02320305	MART-1 Antigen with or without TLR4 Agonist	Glucopyranosyl Lipid A - Stable Oil-in-Water Emulsion (GLA-SE)	Skin Melanoma	Early Phase 1	Mayo Clinic
NCT01418235	SAAVI DNA-C2, SAAVI MVA-C and Novartis Subtype C gp140	MF59	HIV Preventive Vaccine, HIV Seronegativity	1	HIV Vaccine Trials Network
NCT01991561	Plant-made H5 Virus-like-particle	Alhydrogel or Glucopyranosyl-lipid adjuvant in squalene emulsion (GLA-SE)	Respiratory Tract Infections, RNA Virus Infections	2	Medicago

NCT00002204	HIV p24	MF59	HIV Preventive Vaccine, HIV Seronegativity	1	Chiron Corporation
NCT04762680	SARS-CoV-2 Recombinant Protein	AS03	COVID-19	2,3	Sanofi Pasteur,
NCT00912574	Granulocyte-Macrophage Colony-Stimulating Factor	Montanide ISA-51 adjuvant	Melanoma	NA	University of Virginia
NCT01751048	LEISH-F3 (recombinant protein antigen)	GLA-SE (adjuvant), MPL-SE (adjuvant)	Leishmaniasis	1	National Institute of Allergy and Infectious Diseases (NIAID)
NCT01612000	recombinant hemagglutinin (rHA) antigen	oil-in- water adjuvant (SE)	Influenza	1,2	Protein Sciences Corporation
NCT00000832	Recombinant Envelope Protein, HIV-1 SF-2 rgp120 (BIOCINE)	MF59	HIV Infections	1	National Institute of Allergy and Infectious Diseases (NIAID)
NCT03041766	Sm14: recombinant protein produced in yeast	GLA-SE	Schistosomiasis	2a	Oswaldo Cruz Foundation
NCT01147068	recombinant hemagglutinin (rHA) antigen	Glucopyranosyl Lipid A (GLA-SE)	Influenza	1,2	Protein Sciences Corporation
NCT01556945	Falciparum Merozoite Protein-1 (FMP1) and SmithKlineBeecham (SKBB) Candidate Malaria Vaccine RTS,S	SBAS2, oil in water emulsion	Malaria, Falciparum	1,2	U.S. Army Medical Research and Development Command

3.1. Emulsions

Emulsions as vaccine delivery systems are classified based on their active components, such as Mineral oil-based, Saponin-based, and Squalene-based emulsions. Mineral Oil emulsions have been used historically as adjuvants due to their ability to create a depot effect, prolonging antigen exposure to the immune system. Freund first employed the use of mineral oil-based emulsion as an adjuvant, and he developed Complete Freund's adjuvant (CFA) and Incomplete Freund's Adjuvant (IFC) between the 1940s and 1950s (50). Both were w/o emulsions composed of mineral oil with Arlacel-A as an emulsifier. CFA contained killed mycobacteria, while IFC contained an identical formulation but without the killed mycobacterium. Both showed good adjuvant activity but high reactogenicity. This high reactogenicity is thought to occur because of the high viscosity of oil-based formulations. It is suggested that re-emulsifying them in water as w/o/w emulsions would reduce it (51). The focus was initially on developing w/o emulsions as it was thought that it would be more efficient since it forms a depot, but it gave rise to tolerability issues. These included the formulation of Montanide ISA 51 VG, Montanide ISA 720 VG, and Adjuvant 65. All were w/o emulsions composed of a medicinal oil of mineral origin, squalene oil, and peanut oil, respectively (52,53).

Saponins are glycosides derived from plant sources and stimulate strong immune responses. The commercial formulations of Saponins include QS-21 and Matrix-M (54,55). QS-21 is a highly purified saponin often formulated in combination with other adjuvants such as AS01 to enhance its immunostimulatory effects in RTS,S malaria vaccine (55). Matrix-M is a nanoparticle-based adjuvant containing Quillaja saponins have shown promise in enhancing immune responses in various vaccine formulations by promoting both humoral and cellular immunity (54).

Later, squalene-based o/w emulsions developed as emulsion adjuvants with much lower oil content and non-ionic surfactants as emulsifiers. These included MF59, AS03, and AF03. These adjuvants allowed adding the antigen to the preformed emulsion, which meant easier manufacturing and more protection for the antigen from any risk of denaturation during the emulsification process. Moreover, due to their lower oil content, they had better viscosity and syringeability (51). In general, emulsion adjuvants were not found to require physical association with the antigen, but both should be administered concurrently as a mixed formulation (56).

MF59 is a well-known and successful emulsion adjuvant currently used in several subunit vaccines. It showed good immune stimulation, tolerability, and safety profiles. It was able to improve the immunogenicity of influenza, human immunodeficiency virus (HIV), herpes simplex virus (HSV), hepatitis B/hepatitis C virus (HBV/HCV), parvovirus, human papillomavirus (HPV), cytomegalovirus (CMV) vaccines (57). MF59 comprises squalene oil along with Tween 80 and Span 85 nonionic surfactants (58). MF59 only acts as an adjuvant when formulated as an emulsion; no adjuvant effect exists for any of the individual components (59). MF59 was a more potent stimulant of antibody and CD4+ T cell responses than alum (60), but unlike alum and w/o emulsions, it does not act as an adjuvant by forming a depot. Alternatively, it acts by activating immune cells at the injection site in the muscle tissue. Consequently, it produces chemokines, influx of phagocytic cells, and antigen transport to lymph nodes, resulting in an immune response through B and T cell activation and production of antibodies (61,62).

The biodistribution of emulsion adjuvants could be altered by changing their properties, such as size and charge (63). Shah et al. prepared o/w adjuvant emulsions with variable ratios of the MF59 emulsion compositions and with different droplet sizes. It was demonstrated that the droplet size significantly affected adjuvant efficiency with an associated effect on immune cell recruitment and activation. The larger droplet size of 160 nm showed better adjuvant activity than the smaller droplet size of 20 nm (13). In another recent study, emulsions were prepared with different compositions of squalene, surfactant mixtures, and CMC solutions. Some of these formulations induced a better immune response than a commercial alum-based adjuvant upon a second immunization, with the highest immune response exhibited by the formulation composed of 12% squalene with 0.5% ultra-high viscous CMC (64).

The development of emulsion adjuvants remains a researched area to date, with lots of research being done on varying the composition of the emulsion, employing different formulation methods, and incorporating additional stimulators. For the battle with the COVID-19 pandemic, a recombinant SARS-CoV-2 spike antigen was formulated with alum and emulsion adjuvants. Animal studies in mice showed that emulsion adjuvants with a low antigen dose produced a higher humoral immune response and Th1-biased cellular immune responses than alum adjuvants (65). In another study, the RBD spike of SARS-CoV-2 was formulated with an o/w emulsion and a w/o emulsion with squalene. The animal immunization study showed a similar cellular immune response by both adjuvants but an earlier humoral response by the w/o emulsion (66).

A squalene-based emulsion was stabilized by chitosan hydrochloride salt. An enhanced humoral immune response was observed even in comparison to alum, in addition to a potent cellular (Th1-polarized) immune response and increased IFN-γ-secreting splenocytes (67). Terpenoid-based emulsions were also formulated to substitute squalene with other semisynthetic analogs, and some showed an enhanced adjuvant activity (68). In another effort, squalene oil was also replaced by oils extracted from a plant source, Pinaceae-derived polyprenol oils. The nanoemulsions produced comparable physical stability, in vitro cytokine production, and antigen-specific immune responses in animal models to squalene-based emulsions (69). A recent trial was made to convert the MF59 liquid emulsion adjuvant to a dry powder by thin-film freeze-drying (TFFD). This did not alter the immunogenicity of the vaccine in animal studies and would reduce the vaccine's need for cold chain storage and its sensitivity to freezing (70).

AS03 is also composed of squalene oil and Tween 80 surfactant but with an additional oil component, α-tocopherol (vitamin E). This was added for its antioxidant effect to protect the squalene oil from degradation. Still, it also has an immunopotentiation effect as it enhances humoral and cell-mediated immunity (71), which adds to the emulsion adjuvant potency. Its presence results in the direct activation of the innate immunity in the draining lymph node (56). AS03, therefore, enhances antigen uptake by target immune cells and exhibits Th1/ Th2 responses (72). It has been used in H5N1 and H1N1 influenza vaccines (73). The vaccines were well tolerated with mild to moderate adverse effects, but further investigations were required to rule out their association with the incidence of narcolepsy, especially in children (74,75). AF03 is another squalene-based o/w emulsion, utilizing the surfactants montane and eumulgin (76). AF03 had been used as an adjuvant for the influenza split virion vaccine, Humenza, which was licensed but not commercialized (77). Clinical studies showed higher antibody titres resulting from the adjuvanted vaccine in comparison to the non-adjuvanted one, with better antibody persistence (78). Incidences of anaphylaxis following the administration of AS03-adjuvanted H1N1pdm09 vaccine substantially exceeded that reported with seasonal influenza vaccines (79). In addition, water-in-oil emulsion adjuvants can also denature the structure of emulsified protein antigens due to their hydrophobic nature, potentially disrupting both hydrophobic and electrostatic interactions (80,81).

Host-derived damage-associated molecular patterns such as host DNA (DAMPs) adjuvants such as Alum alone cannot induce protective type-1 (cellular) immunity, including the induction of Th1 cells and the activation of cytotoxic T lymphocytes, NK cells, and phagocytes. A potent pathogen-

associated molecular pattern (PAMP) adjuvants such as Toll-like receptor (TLR) agonists are often required to induce cellular type 1 immune response (82). Emulsion adjuvants enable the use of lower antigen doses and quicker immune responses by creating an "immunocompetent environment" at the injection site, followed by robust and long-lasting germinal center responses in the draining lymph nodes. Consequently, emulsion adjuvants trigger distinct immunological reactions, including a mixed Th1/Th2 T cell response, long-lived plasma cells, a broader range of memory B cells, and high levels of cross-neutralizing polyfunctional antibodies against viral variants. A recent study compared the adjuvant effects of alum and MF59 emulsion. The emulsions showed stronger IgG2b and IgG2c responses by potentiating helper Th1 and TFH cell activity and higher antigen-specific CD8 T cell responses in lymph nodes and non-lymphoid tissues (83). Due to these properties, MF59 and AS03 were included in the influenza vaccines used during the 2009 H1N1 influenza pandemic and are still part of seasonal influenza vaccines (84).

A novel vaccine adjuvant based on an oil-in-water emulsion of squalene oil with aqueous glucopyranosyl lipid (GLA, TLR4 ligand) improves TFH responses resulting from the combined activity of the emulsion and the stimulation of TLR4 (85). GLA-SE was combined with split-virus vaccines to stimulate cytotoxic T lymphocyte (CTL) responses and to enhance influenza vaccine efficacy in older adults for clinical protection against influenza (86). GLA was further optimized as the second-generation lipid adjuvant (SLA), a synthetic hexa-acylated lipid for activation of the human TLR4/MD2 receptor complex (87). Knudsen et al. conducted a head-to-head comparison of MF59®, GLA-SE with other adjuvants, Alum, IC31®, and CAF01, using antigens from M. tuberculosis, influenza, and chlamydia in mice. irrespective of the antigen used. MF59® elicited strong antibody and IL-5 responses, while GLA-SE promoted antibodies and Th1 responses and were particularly effective in inducing influenza HI titers. At the same time, CAF01, GLA-SE, and IC31® enhanced protection against TB and chlamydia. The results suggest that each adjuvant induced a unique immune response and has the potential for different disease targets, providing a foundation for the rational development of next-generation vaccines for human use (88).

Even though, emulsions have shown promising vaccine delivery vehicles, several real-world barriers exist. One of the primary challenges is the stability of emulsion adjuvants, which are sensitive to freezing, limiting their use in areas with unreliable cold-chain infrastructure (89). Recent advances, such as thin-film freeze-drying (TFFD) of emulsions, have shown promise in creating thermostable formulations, but these technologies require further optimization for large-

scale production (90). Use of emulsion adjuvants in clinical settings also highlights the need for rigorous safety evaluations. For example, AS03-adjuvanted H1N1 vaccines were associated with a possible increased risk of narcolepsy in certain populations, which underscores the importance of post-marketing surveillance to identify rare adverse events (74). Moreover, the viscosity and syringeability of some emulsion formulations can complicate large-scale immunization programs (91).

3.2 Liposomes

Liposomes gained interest as a delivery system for drugs and vaccines. The employment of liposomes for vaccines dates back to the 1970s when diphtheria toxoid was entrapped into liposomes, and a higher antibody concentration was observed in mice following their immunization with those liposomes in comparison to immunization with the free toxoid (92). As adjuvants, liposomes function as both immunopotentiators and delivery systems for subunit antigens (93). This was supported by their structural similarity to cellular membranes, biocompatibility, flexibility to be administered through different routes, capability to carry different moieties of both hydrophilic and lipophilic natures either in the aqueous core or within the lipid bilayer respectively, as well as their ability to deliver them to APCs (93)(94,95). In addition to carrying compounds within their interior, liposomes could also accommodate compounds attached to their surface by electrostatic or covalent interactions. Furthermore, liposomes control the release of drugs or antigens with their capability to form a depot to prolong the activation of APCs, protect them from degrading in-vivo conditions, increase their stability, alter their biodistribution, and enhance their bioavailability as well as efficacy (2,14,15,96–98).

Despite these advantages, liposomes face practical challenges in real-world application. One significant barrier is their cost. The production of liposomes involves complex manufacturing processes, such as microfluidics and nanoprecipitation, which are not always scalable or cost-effective (99,100). Additionally, the stability of liposomal formulations remains a concern, particularly in terms of maintaining their physicochemical properties during storage and transport (101).

In clinical use, the surface charge and size of liposomes influence their interaction with the immune system. For example, cationic liposomes show enhanced immune activation but may also induce

cytotoxicity at higher doses (102,103). Meanwhile, achieving an optimal balance between liposome stability and immune activation requires careful formulation, as liposomal rigidity affects depot formation and antigen release (104).

Another barrier is regulatory complexity. Liposomal vaccines often require extensive preclinical and clinical testing to demonstrate safety and efficacy due to the variability in immune responses caused by different lipid compositions (105). This can delay their approval and widespread adoption.

Factors Affecting Adjuvant Properties

Table 3 summarizes the factors affecting the adjuvant properties of liposomes and emulsions.

Table 3: The optimization of factors affecting the adjuvant properties of liposomes and emulsions for an enhanced immune response.

Factor affecting adjuvant properties	Optimization
Vesicle Size	• Liposomes with a smaller size (up to 100 nm) result in a high lymphatic uptake, while those with a larger size slow the lymphatic uptake and increase the retainment in the interstitial spaces (106). • Larger emulsion droplets (~160 nm) result in better recruitment of immune cells to the site of injection compared to smaller droplets (~ 20 nm) which in turn lead to greater antigen uptake, faster translocation to draining lymph nodes (dLN) and improved cellular and humoral responses (13). • DCs endocytose smaller liposomes (20-200nm), while macrophages mostly phagocytose larger liposomes (> 500nm) (94,95). • Larger liposomes are better retained at the regional lymph nodes than the smaller ones, which migrate faster to the bloodstream (107). • Reducing the size of liposomes improves both humoral and cellular immune responses (108). • Larger liposomes exhibit a more depot effect and allow more gradual release (95). • Smaller liposomes better stimulate the Th2 response than the Th1 response and vice versa (109,110).
Composition	• Biodegradable oils like squalene improve safety and immunogenicity (66). • Both W/O and O/W emulsions have strong adjuvant effects, O/W emulsions exhibit better safety and tolerability (111). • Cholesterol reduces the lipid bilayer's permeability as it results in a dense packing of the phospholipids, making liposomes more stable (14). Increasing cholesterol also results in a higher humoral response (112). • Liposomes composed of unsaturated fatty acids have their antigens processed at MHC I and MHC II and show higher uptake by APCs. In contrast, liposomes of saturated fatty acids have most of their antigens processed at only MHC II and show less uptake by APCs (113). • Immunogenicity increases with decreasing fluidity. The rigid liposomes can form a depot and thus have prolonged interaction with APCs, while fluid liposomes do not support depot formation as they rapidly clear from the injection site (104). • Liposomes composed of lipids with an intermediate Tm (30-41 °C) exhibit a better immune response than those composed of lipids with high or low Tm (112).
Surface Charge	• Neutral liposomes are the least immunogenic, while cationic liposomes are the most immunogenic (102,103). Same is observed for emulsions (114).

	• Charged liposomes interact more with cells, and cationic liposomes have the highest endocytosis rate (115,116). • Cationic liposomes are better at forming depots at the injection site, which could prolong the vaccine's exposure to the immune cells (117,118). • Anionic liposomes drain faster to the lymph nodes due to their minimal interaction at the site of action (119). • Neutral liposomes are less stable, as the surface charge of liposomes prevents their aggregation and enhances their stability (15).
Antigen Association	• Antigen and adjuvant association is necessary for eliciting an immune response rather than their co-administration (16). • The form of antigen-adjuvant association influences their adjuvancy. Surface-associated antigens produce a better humoral response than encapsulated ones (120). • Stronger antigen-to-liposome adsorption results in a better depot effect (117).
Liposomes Modification	• PEG-coated liposomes are long-circulating, as PEG prevents their opsonization and clearance. It also supports the passive targeting of liposomes by avoiding the MPS uptake (121). • pH-sensitive liposomes present specific targeting and are designed to release their loaded drug only in response to a specific pH trigger (122). This strategy utilizes that some pathological tissues, like tumors and infected areas, are generally acidic (123). • Immunoliposomes contain attached antibodies or antibody fragments specific to their target antigens or receptors and thus support active targeting (124). • Attaching different ligands to liposomes, such as peptides, carbohydrates, glycoproteins, receptor ligands, and growth factors, improves the active targeting of liposomes to selective target cells (15). • The incorporation of immunostimulatory components such as bacterial-derived glycolipids, nucleotide-based molecules, or TLR agonists that could activate PRRs produces enhanced immune system activation (2). • Fusogenic liposomes are designed to fuse with the cell membranes of APCs and deliver their loaded compounds directly into their cytoplasm (2).

Tm: Melt transition temperature

3.2.1. Vesicle Size

The vesicle size is an essential factor determining the adjuvant activity. It influences its drainage from the site of injection, as a vehicle with a large size might not be able to pass to the lymphatic ducts and might remain at the site of injection. The ideal size for fast and high lymphatic uptake

would be up to 100 nm, above which the uptake becomes slower with more chance of retaining in the interstitial spaces (106). However, lymphatic uptake was observed for particles as large as 1 μm (125). This effect of size, in turn, alters the immune response. It was shown that reducing the size of cationic liposomes improved both humoral and cellular responses (108). Additionally, smaller vesicles stimulated the Th2 response better than the Th1 response, which was increased by vesicles of larger size (109,110). DCs endocytose smaller liposomes (20-200nm), while macrophages mostly phagocytose larger liposomes (> 500nm) (94,95). A supporting in-vitro model showed the uptake of particles by dendritic cells being optimal, with particles having a size of not more than 500 nm (126). Upon SC injection, lymphatic uptake was higher for the smaller liposomes than the larger ones retained at the injection site. However, the larger liposomes were better retained at the regional lymph nodes than the smaller ones, which migrate faster to the bloodstream (107). Moreover, they exhibit a more depot effect and allow more gradual release (95).

3.2.2. Surface Charge

Liposomes could be termed neutral, anionic, or cationic based on their surface charge. Neutral liposomes exhibited the least immune response, while cationic liposomes were the most immunogenic (102,103). This could be attributed to the better ability of charged liposomes to associate with cells, which was observed in an in-vitro study. Cationic, anionic, and neutral liposome uptake was studied in a human ovarian carcinoma cell line (HeLa) and a murine-derived mononuclear macrophage cell line (J774). Cationic liposomes showed a significantly higher endocytosis rate by HeLa cells than neutral and anionic ones. In contrast, cationic and anionic liposomes interacted more with J774 cells than neutral ones (115). Similarly, the interaction of positively charged particles with the negative cell membranes supports their endocytosis and clearance (116).

It is also thought that positively charged liposomes are better at forming depots at the injection site, possibly by their interaction with the negatively charged cells and proteins. This depot effect could prolong the vaccine's exposure to the immune cells (117,118). Although particles with a large size of 1 μm do not seem to show high cellular uptake, they still show a better uptake if they have a positive surface charge (126). On the other hand, liposomes with a negative charge drain faster to the lymph nodes due to their minimal interaction at the site of action (119). Regarding

physical stability, neutral liposomes aggregate more, making them less stable as a surface charge supports their repulsion and renders them more stable (15). However, the concern with cationic liposomes is their cytotoxicity (127).

Araujo et al. formulated a cationic liposome formulation CAF01 and employed it as an adjuvant for the peptide P10, a candidate vaccine for Paracoccidioidomycosis, a systemic fungal infection. Mice infected with the fungus were vaccinated with the liposomal formulation, and an effective cellular immune response was observed with an enhanced antifungal potency (128). In another study, cationic liposomes composed of the cationic lipid dimethyl dioctadecylammonium bromide (DDAB), cholesterol, and oleic acid were produced to encapsulate an anti-leishmanial antigen. When IM injections were given to mice, DC functional maturation resulted, and liposomes were drained to lymph nodes, accompanied by the production of antigen-specific immunoglobulins and T cells (129). Moreover, another study involved the preparation of a liposomal vaccine adjuvant incorporating S-lactosylarchaeol glycolipids. Several protein antigens were added to the formulations by encapsulating or admixing with the liposomes. All formulations prepared by both antigen association techniques induced strong humoral and cell-mediated antigen-specific immune responses in mice (130). Mullertz et al. modified the CAF01 liposomal adjuvant incorporating the subunit antigen H56 for the TB vaccination by substituting the cationic lipid dimethyldioctadecylammonium (DDA) with other lipids and changing the surface charge. A better antigen-specific cellular immune response was observed (131). Furthermore, Tada et al. developed a nasal vaccine using cationic liposomes with the model antigen ovalbumin (OVA) and explored its immunological effect following the intranasal immunization of mice. Antigen-specific nasal immunoglobulin A (IgA) and serum immunoglobulin G (IgG) were produced only in mice who received the antigen with the adjuvant, unlike the mice who received only the antigen and showed no production of immunoglobulins. The adjuvant vaccine formulation also induced strong IL-6 expression at the administration site (132).

3.2.3. Lipid Composition and Bilayer Fluidity

Individual lipids have a characteristic phase transition temperature (Tm), above which they transform to the fluid phase, where they become in a liquid crystalline state. In contrast, the lipid bilayers are in a solid gel-like state below this temperature. This Tm depends on the length and

saturation degree of the hydrocarbon chains as well as the packing of the lipids. Longer acyl chains allow stronger interaction and, thus, less lipid mobility. Similarly, the more saturated lipid chains offer less free space and flexibility. Both factors give rise to higher Tm. Therefore, the lipid composition of liposomes alters their properties. Incorporating cholesterol with the lipids stabilizes the vesicles by reducing the lipid bilayer's permeability, resulting in a dense packing of the phospholipids. High cholesterol concentrations could eliminate the phase transition and reduce the fluidity of liposomes above the Tm, thus making them more stable (14). Increasing cholesterol also results in a higher humoral response (112). Furthermore, cholesterol also acts as an anchor for some molecules attaching to liposomes, such as PEG or deoxyribonucleic acid (DNA) (133).

The fluidity of liposomes is a considerable factor relating to their adjuvant properties. Liposomes with a Tm below 37 °C will be fluid at body temperature. When observed in-vitro, liposomes composed of unsaturated fatty acids had antigens processed at both MHC I and II and were uptaken by APCs, unlike liposomes of saturated fatty acids, which had most of their antigens processed at only MHC II and were not up taken by APCs (113). However, a much stronger Th1 immune response was observed with rigid liposomes than highly fluid ones. Generally, immunogenicity increases with decreasing fluidity. The rigid liposomes can form a depot and thus have prolonged interaction with APCs, while fluid liposomes do not support depot formation as they rapidly clear from the injection site (104). Liposomes composed of lipids with an intermediate Tm (30-41°C), such as dipalmitoyl L-α-phosphatidylcholine (DPPC), seemed to exhibit a better immune response than those composed of lipids with high or low Tm like distearoyl L-α-phosphatidylcholine (DSPC) which has a Tm of 58 °C or dilauroyl L- α -phosphatidylcholine (DLPC) with a Tm of 0 °C. Increasing cholesterol was also directly related to a higher humoral response (112).

3.2.4. Antigen association

Liposomes could incorporate antigens in different ways, either entrap them in their internal aqueous environment, embed them in their bilayer membrane, or associate them to their surface, possibly by electrostatic interactions as with cationic liposomes. The antigen-adjuvant association could also influence their adjuvancy. In previous research, it had been reported that although both liposomes with surface-associated and encapsulated peptides produced cellular immune responses, only the surface-associated liposomes could produce a humoral response, but not the encapsulated ones. In contrast, the mixture of peptides with free liposomes failed to elicit any immune response

(120). Moreover, the stronger antigen to liposome adsorption resulted in a better depot effect (117). Whether the liposomes are associated with the antigen or not can have different effects on immune functions (134). Several studies suggested the importance of the antigen association with the liposomes for its adjuvant effect. Administering the antigen and adjuvant separately at the same site prevented the Th1/Th17 responses that resulted from administrating the same antigen and adjuvant co-formulated and associated (16). This association was shown to be of particular importance for the primary humoral response and was shown to trigger a more rapid and sustained antibody production (134).

Many studies suggest the effect of antigen absorption on the depot-forming adjuvants on humoral immune responses (135–137). T-cell responses require costimulatory signals typically provided by antigen-presenting dendritic cells migrating from the injection site. Therefore, antigen delivery alone is not sufficient for inducing effector T cells, and co-localization of the antigen and the adjuvant is required (16). In one study, administering non-adsorbed antigen with the depot-forming, cationic liposome-based adjuvant CAF®01 results in low T cellular responses, while adsorbed antigen complements T mobile responses, particularly benefiting Th17 responses (138). A recent study confirms the finding of this investigation (16). Both the amount (139) and the duration of retention (140) of Antigens at the injection site is essential for inducing strong effector T cell responses; therefore, absorption of antigen to the adjuvant should be the essential consideration in the vaccine design.

3.2.5. Liposome Modifications

Liposomes could be classified according to their properties into conventional liposomes, pH-sensitive liposomes, immunoliposomes, and long-circulating liposomes (*Fig. 1*).

Conventional liposomes are usually phagocytosed by the circulating macrophages of the RES (14). Coating the surface of liposomes with a shielding polymer such as polyethylene glycol (PEG) is one strategy to produce long-circulating liposomes known as stealth liposomes. This reduces their surface charge, prevents their opsonization and clearance, and prolongs their circulation time. It also supports the passive targeting of liposomes because they reach their target site by avoiding the mononuclear phagocytic system (MPS) uptake (121). However, attempts have been made to allow the shedding of this polymer coating at the target site to allow desirable drug release and cellular interaction (116). The surface PEGylation of liposomes does not only alter the

biodistribution of liposomal adjuvants but also the immune response. 10% PEG with SUVs resulted in an earlier antibody response and shifting from a Th1 to a Th2 response (118).

pH-sensitive liposomes show promise in delivering peptides for use as prophylactic or therapeutic vaccines. pH-sensitive liposomes present specific targeting and are designed to release their loaded drug only in response to a specific pH trigger. They are generally stable at the physiological pH, but when encountering acidic conditions, they destabilize and release their loaded compounds (122). This strategy utilizes some pathological tissues, like tumors and infected areas, which are typically acidic (123). Ovalbumin-containing modified liposomes with a pH-sensitive polymer, succinylated poly(glycidol) prepared by Watarai et al. resulted in significantly higher levels of ovalbumin-specific IgG3, IgG2a, and IgG1 antibodies compared to unmodified liposomes in mice (141). Chang et al. developed pH-sensitive liposomes encapsulating V3-loop peptide, a key component of HIV vaccines. The study demonstrated that the liposomes elicited cytotoxic T-lymphocytes (CTL) and virus-specific neutralizing antibodies, whereas no response was observed without liposome encapsulation (142). Lee et al. investigated the immunization potential of pH-sensitive liposomes containing fluorescein isothiocyanate-conjugated H-2Kb CTL epitope, showed significant activation of CTL responses after three days of immunization (143).

Active targeting is also exhibited by immunoliposomes, which are liposomes with attached antibodies or antibody fragments and are specific for their target antigens or receptors (124). Similarly, other ligands are also used to actively target liposomes to selective target cells, such as peptides, carbohydrates, glycoproteins, receptor ligands, and growth factors (15). Immunoliposomes containing monoclonal antibodies (MAbs) linked to rgp120-containing liposomes through a biotin–avidin–biotin bridge were accessed for targeting costimulatory molecules CD28 and CTLA4, along with their counterreceptors B7-1 (CD80) and B7-2 (CD86), to enhance the immune response to recombinant envelope protein rgp120 of the MN strain of human immunodeficiency virus type 1 (HIV-1). Mice vaccinated with immunoliposomes showed Mab-dependent robust delayed-type hypersensitivity response to the weakly immunogenic gp120, without provoking a humoral immune response (144).

Liposomes could also be modified to produce enhanced immune system activation. This could involve incorporating immunostimulatory components such as bacterial-derived glycolipids, nucleotide-based molecules, or TLR agonists that could activate PRRs (2). The liposome-based

adjuvant system AS01 is a proprietary adjuvant system (licensed by GSK from Antigenics Inc., a wholly owned subsidiary of Agenus Inc., a Delaware, USA corporation) contains two immunostimulants: MPL and QS-21. MPL (3-O-desacyl–monophosphoryl lipid A) is a detoxified derivative of lipopolysaccharide from Salmonella Minnesota and stimulates activation of innate immunity via Toll-like receptor 4 (TLR4). QS-21 is an amphiphilic triterpene glycoside that activates specific innate pathways in monocytes (i.e., the ASC-NLRP3 inflammasome, a multi-protein complex leading to the release of IL-1β/IL-18). Both MPL and QS-21 are required to achieve the highest antigen-specific adaptive response. QS-21 contributes the most to the antibody response, and the addition of MPL greatly enhances this response. Combining both was essential to elicit varicella-zoster virus glycoprotein E antigen-specific polyfunctional CD4+ T-cell response in herpes-zoster vaccine, Shingrix® (55). AS01 is present in Arexvy, the first approved vaccine which elicits broad neutralization of contemporary and antigenically distant respiratory syncytial virus strains (145). AS01 showed increased efficacy, T helper type 1 (TH1) cell–mediated immunity, and antigen-specific humoral immunity in both mice and humans compared to when same immunostimulants (MPL and QS-21) were formulated in an oil-in-water emulsion (146). A SARS-CoV-2 spike subunit vaccine was formulated using a dual TLR ligand liposome adjuvant. The vaccine showed a high protective efficiency in a mice model exposed to a lethal SARS-CoV-2 challenge. It induced systemic and local anti-Spike IgA antibodies, and two immunizations showed protection from the lung injury that occurred in the control mice (147). Recently, a study was done to determine prophylaxis from future respiratory virus pandemics. A liposome-based vaccine adjuvant, CAF09b, containing a TLR3 agonist, was formulated. Testing for prophylaxis in mice activated the innate immune system and IFN-I gene expression responses. When administered before challenging the mice with the influenza virus, the virus was still detectable, but it reduced the severity of the disease (148).

Liposomal adjuvant formulations combined with PRR agonists were developed for tuberculosis subunit vaccines based on recombinant CMFO protein. The developed formulations resulted in strong and long-lasting immune responses (149). Lathrop et al. developed cationic liposomes with TLR4 agonist for the subunit vaccine of SARS-CoV-2 Spike protein and studied the effect of liposomes composition and charge. Humoral and cellular immune responses were produced in mice, but higher boosting of anti-spike antibody titers resulted from charged liposomes than neutral liposomes (150). Furthermore, recombinant subunit antigens of Enterotoxigenic

Escherichia coli were formulated with adjuvant liposomes. Systemic and mucosal immune responses were studied following the IM immunization of mice. Serum IgG and intestinal IgA antibodies were elicited. In-vitro studies also showed enhanced delivery of the antigens to macrophages (151).

Fusogenic liposomes were also explored. Those liposomes are designed to fuse with the cell membranes of APCs and deliver their loaded compounds directly into their cytoplasm (2). To confer fusogenic properties to liposomes, Dioleoyl phosphatidylethanolamine (DOPE) and cholesteryl hemisuccinate (CHEMS) are mostly used (152). The extent of internalization, fusogenic ability, and stability in biological fluids of fusogenic liposomes are determined by the selection of amphiphilic stabilizers and their molar percentage with regard to the lipids (153). Poly(glycidol) derivatives such as 3-methylglutarylated poly(glycidol) (MGlu-PG) and succinylated poly(glycidol) (Suc-PG) have been studied for liposome modification due to their fusogenic properties at mildly acidic pH. These modified liposomes, with carboxyl groups in the polymeric side chain, are preferentially taken up by dendritic cells, leading to efficient CTL activation (154). Yuba et al. prepared ovalbumin-loaded pH-sensitive liposomes modified with MGlu-PG of linear (MGlu-LPG) and hyperbranched structure (MGlu-HPG). The modified liposomes induced stronger OVA-specific cellular immune responses and tumor suppression in 50–75% of mice upon subcutaneous or nasal administration (155). MGlu-HPG forms more hydrophobic domains under weakly acidic conditions than MGlu-PG, enhancing its membrane disruption ability. The fusogenic property of MGlu-HPG increases with polymerization degree, enabling efficient recognition by scavenger receptors on dendritic cells (156).

4. Conclusion

Subunit vaccines are safer than conventional live attenuated and inactivated vaccinations since they only use purified antigens. Subunit antigen delivery presents several difficulties, one of which is their decreased effectiveness, which calls for adding adjuvants. The type, nature, and size of the adjuvant employed, the physicochemical properties of the delivery system, and the method of vaccine administration all affect how immunogenic the vaccine is. The application of lipid-based delivery systems, specifically emulsions and liposomes, in vaccine development represents a significant advancement in addressing the limitations of subunit vaccines. These systems enhance

antigen presentation and immune activation, providing a promising pathway to achieving robust and durable immunity. Their potential impact on diagnosis, treatment guidelines, vaccine efficacy, and healthcare economics is profound, offering both immediate and long-term benefits for public health.

5. Expert Opinion

Lipid-based delivery systems, such as emulsions and liposomes, represent a paradigm shift in vaccine technology by enabling robust humoral and cellular immune responses with lower antigen doses, a property that is particularly critical during pandemics or in resource-limited settings. These adjuvants can optimize vaccine administration strategies by potentially reducing the frequency of booster doses, thereby improving patient compliance and lowering healthcare costs. While emulsions excel in dose-sparing and broadening immune responses, liposomes offer customization and precision in antigen delivery.

However, the broader clinical application of these technologies is not without challenges. Stability issues, such as the susceptibility of emulsion-based adjuvants to freezing and their reliance on cold-chain logistics, pose significant barriers to their use in remote or underserved regions. Developing dry powder formulations or other thermostable formats could mitigate this challenge and enhance global vaccine equity. Similarly, while squalene's biodegradability and biocompatibility make it a preferred choice for oil-in-water emulsions, its extraction from natural sources like sharks raises ethical and sustainability concerns. Advances in synthetic or plant-derived oils as alternatives are vital to addressing these issues.

The versatility of lipid-based adjuvants offers several promising avenues. For instance, the ability to customize liposomal size, surface charge, and lipid composition allows for tailored immune responses targeting specific populations, such as the elderly or immunocompromised individuals. This adaptability aligns with the increasing emphasis on personalized medicine and precision vaccination. Additionally, the incorporation of immunostimulatory molecules into lipid-based systems could further enhance their efficacy, especially in developing vaccines for challenging pathogens like HIV or tuberculosis.

Future developments in this field will likely focus on improving manufacturing scalability and cost-effectiveness. Techniques such as microfluidic production and high-throughput screening of lipid formulations could streamline the development and deployment of lipid-based adjuvants. Moreover, leveraging novel delivery routes, such as intranasal or transdermal administration, may broaden their clinical applications and facilitate mass immunization during pandemics.

Funding:

This paper was not funded.

Declarations of Interest:

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

Reviewer Disclosures:

Peer reviewers on this manuscript have no relevant financial or other relationships to disclose.

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Tables and figures:

Table 1: FDA-approved subunit vaccines (12)**.**

Table 4: Emulsion and Liposome-Based Adjuvants in Clinical Trials (accessed on 28th November 2024).

Table 3: The optimization of factors affecting the adjuvant properties of liposomes and emulsions for an enhanced immune response.

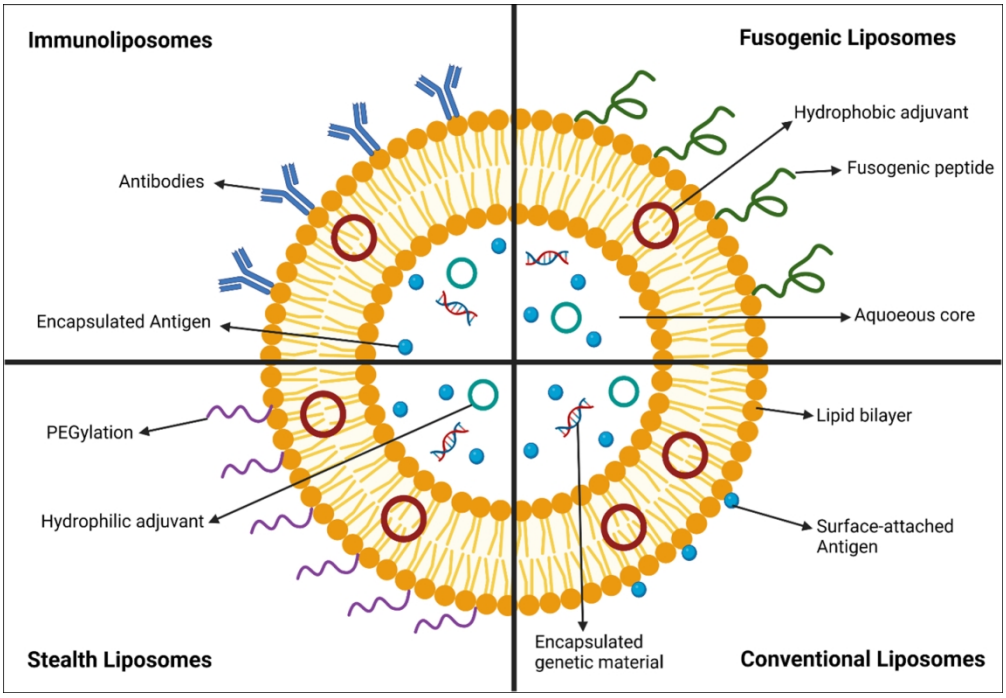

Fig. 1: Modifications of Liposomes as vaccine adjuvants

418x288mm (87 x 87 DPI)