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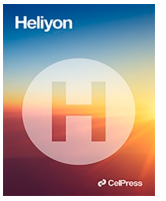

Review article

Research progress on emulsion vaccine adjuvants

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ABSTRACT

Vaccination is the most cost-effective method for preventing various infectious diseases. Compared with conventional vaccines, new-generation vaccines, especially recombinant protein or synthetic peptide vaccines, are safer but less immunogenic than crude inactivated microbial vaccines. The immunogenicity of these vaccines can be enhanced using suitable adjuvants. This is the main reason why adjuvants are of great importance in vaccine development. Several novel human emulsion-based vaccine adjuvants (MF59, AS03) have been approved for clinical use. This paper reviews the research progress on emulsion-based adjuvants and focuses on their mechanism of action. An outlook can be provided for the development of emulsion-based vaccine adjuvants.

1. Introduction

Adjuvants are non-specific immune enhancers that enhance humoral immunity to a target antigen or alter the type of immune response when injected into the body simultaneously with or in advance of a specific antigen. Adjuvants have long been recognized as an effective means of enhancing the immunogenicity of inactivated influenza vaccines. Adjuvants can strongly enhance the immune responses to vaccines. Quantitative adjuvant enhancement results in an increased antibody response compared with the same dose of vaccine antigen without an adjuvant. An effect known as antigen dose retention means that a similar immune response can be produced using a smaller dose of the antigen [1,2].

Depending on their composition, emulsions can be classified into basic and compounded types. The basic types include oil-in-water (O/W) and water-in-oil (W/O) emulsions (Table 1), whereas the compound types include water-in-oil-in-water (W/O/W) and oil-in-water-in-oil (O/W/O) emulsions. Emulsions can be classified as ordinary emulsions, submicroemulsions, or nano-emulsions, based on the size of the emulsion droplets [3].

Although both W/O and O/W emulsions have strong adjuvant effects, O/W emulsions exhibit better safety and tolerability [4]. In addition, from a formulation point of view, W/O emulsions require emulsification of the antigen solution in water with an oil phase. O/W emulsions have the advantage of allowing an antigen solution in water to mix with a preformed emulsion. This allows the antigen and adjuvant to be stored separately and combined at the point of use before administration. This is particularly important in the

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Table 1
Main components of the emulsion adjuvant.

Type of emulsion	Name of emulsion	Formulation	Examples of vaccine
water-in-oil (W/O) emulsion	Freund's adjuvant	Paraffin Oil; Lanolin; (*Mycobacterium tuberculosis*)	Veterinary vaccine [11]
oil-in-water (O/W) emulsions	MF59	Squalene; Citrate buffer; Tween 80; Span85	Flu vaccine [12] (Fluad®)
	AS03	Squalene; α-Tocopherol; PBS; Tween 80	Malaria vaccine [11,13]; Flu vaccine [14,15] (Pandemrix®、Arepandix®)
	AF03	Squalene; PBS; Span 85; Poly-oxyethylene cetyl ether	Flu vaccine [16] (Humenza)
	Pickering emulsion	Squalene; water/Citrate buffer; Aluminium	Malaria vaccine [17]; COVID-19 vaccine [18]

context of the pandemic prevention program for COVID-19 pneumonia as it allows the storage of large quantities of adjuvants. They can then be easily mixed with antigens prepared from any emerging pandemic strain.

The mechanism of action of emulsion-based adjuvants is not fully understood, but recent advances in immunobiological research have revealed several underlying mechanisms of adjuvant action [5]. Based on the available evidence, immune adjuvants elicit an immune response through one or more of the following mechanisms: sustained release of antigen at the injection site (reservoir effect); upregulation of cytokines and chemokines; recruitment of immune cells at the injection site; increased antigen uptake and presentation to antigen-presenting cells (APCs); activation and maturation of APC cells (increased expression of major tissue compatible complex MHC II and co-stimulatory molecule expression); APC processing and exposure to an antigen; promotion of APC migration to draining lymph nodes; migration of mature APC to draining lymph nodes and interaction with antigen-specific B/T lymphocytes to activate potent antibody production or cytokine secretion [6–10].

2. Classification and mechanism of emulsion-based adjuvants

2.1. Water-in-oil adjuvant: Freund's adjuvant

The use of Freund's adjuvant in experimental and commercial influenza vaccines began with the development of the first water-in-

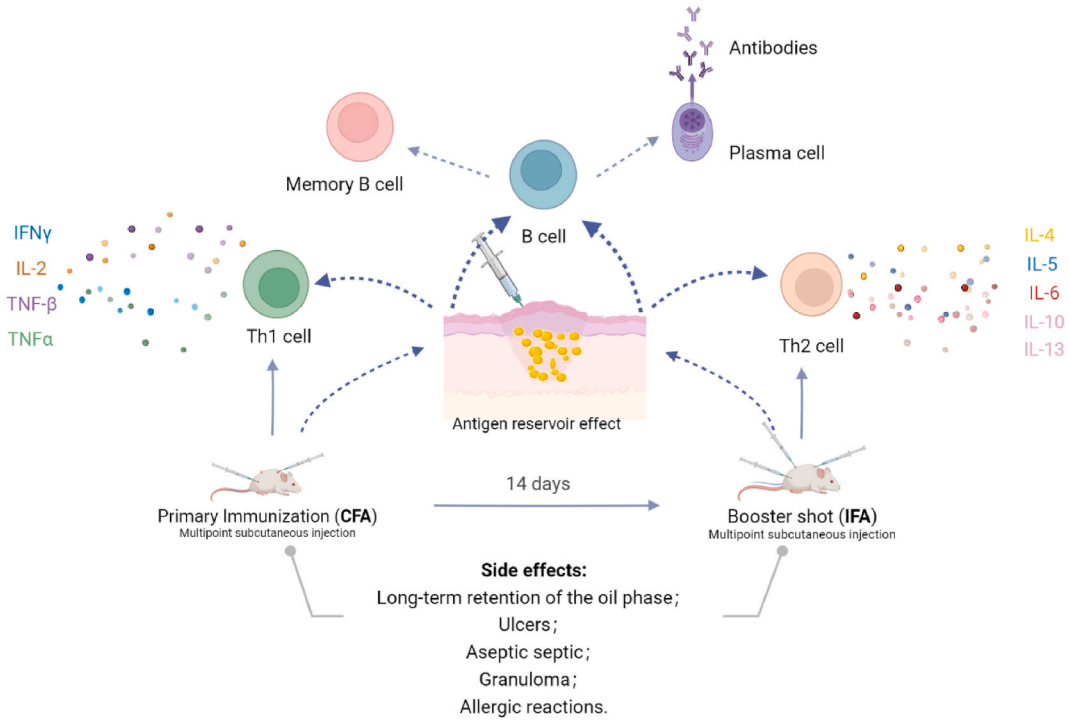

Fig. 1. Mechanism of action of Freund's adjuvant. Subcutaneous injection of Freund's adjuvant triggers an efficient humoral immune response. First immunisation with complete Freund's adjuvant (CFA) induces the production of Th1-type cytokines (e.g. IL-2, INF-γ, TNF-α, TNF-β, etc.) and mediates specific cellular immune responses. Booster shot with incomplete Freund's adjuvant (IFA) induces the production of Th2 type cytokines (e.g. IL-4, IL-5, IL-6, IL-10, IL-13, etc.). However, the main side effects after inoculation with Freund's adjuvant are prolonged residence of the oil phase, ulcers, aseptic septic, granulomas, and a tendency to trigger allergic reactions.

oil adjuvant by Freund in the 1940s. It is a stable water-in-oil emulsion prepared from paraffin oil and lanolin, in which complete Freund's adjuvant (CFA) contains *Mycobacterium tuberculosis*, and incomplete Freund's adjuvant (IFA) does not contain tuberculosis *Mycobacterium* [19]. CFA can significantly enhance humoral and cellular immunity, induce the production of Th1 cytokines (IL-2, INF-γ, TNF-α, TNF-β, etc.), and mediate specific cellular immune responses. IFA induces the production of Th2 cytokines (IL-4, IL-5, IL-6, IL-10, and IL-13). These cytokines activate B lymphocytes and mediate antibody-based humoral immunity [20,21](Fig. 1).

Regarding the mechanism of action, Freund proposed the following three possibilities: the formation of an antigen reservoir at the injection site; efficient transport of antigens to the lymphatic system and lungs, where the adjuvant can promote the accumulation of cells associated with the immune response [22]; and some other mechanism, as yet unidentified [19,22]. Vaccines containing CFA are generally used as primary vaccines, whereas those containing IFA are generally used as adjuvant vaccines to avoid allergic reactions cause by *Mycobacterium tuberculosis* proteins.

Compared with the aluminium adjuvant, the first human vaccine adjuvant, Freund's adjuvant is superior in improving antibody titres and immunity persistence. However, it can easily cause serious adverse reactions such as long-term retention of the oil phase in the tissue after injection. Adverse reactions are difficult to metabolise and excrete. It can cause ulcers at the injection site, sterile suppuration, granulomas, and easy to cause allergic reactions [5,23] (Fig. 1). Therefore, Freund's adjuvant can presently only be used for animal immunisation.

IFA has an improved safety profile compared with CFA, but its efficacy as an adjuvant is unsatisfactory. This has led to the development of improved versions of IFA: Montanide ISA 51 (containing mannitol monooleate) and Montanide ISA 720 (containing squalene) [21]. The combination of Montanide ISA 51 with an HIV- derived peptide antigen mixture (a mixture containing B- and T-cell peptide epitopes) has been shown to enhance the immunogenicity of antigenic peptides. However, these vaccine candidates have failed to meet the safety requirements in clinical trials. Healthy adults who develop stronger antibody responses are more likely to develop serious systemic or local adverse reactions [24]. Candidate vaccines are also more likely to cause serious systemic or local adverse reactions in healthy adults. When Montanide ISA 720 was combined with the malaria vaccine candidate, it induced a higher humoral immune response than the alum-derived adjuvant. Phase 1 clinical trials of Montanide ISA 720 formulated with the peptide antigen showed that it stimulated an effective immune response, although numerous local reactions, such as granulomas, tenderness, and erythema, have also been observed [25]. There is also a new water-in-oil adjuvant formulation called NH_2, whose main components are mineral oil and dehydrated sorbitan monooleate. NH_2 is widely used as an adjuvant for peptide-based cancer vaccination and is superior to Montanide ISA 51 in inducing cellular immune responses in mice [26]. The effect of the vaccine with NH_2-adjuvanted peptide was confirmed in a phase 1 clinical trial in patients with advanced cancer. It effectively increased the secretion of specific IgG antibody and IFN-γ in inoculated patients [27].

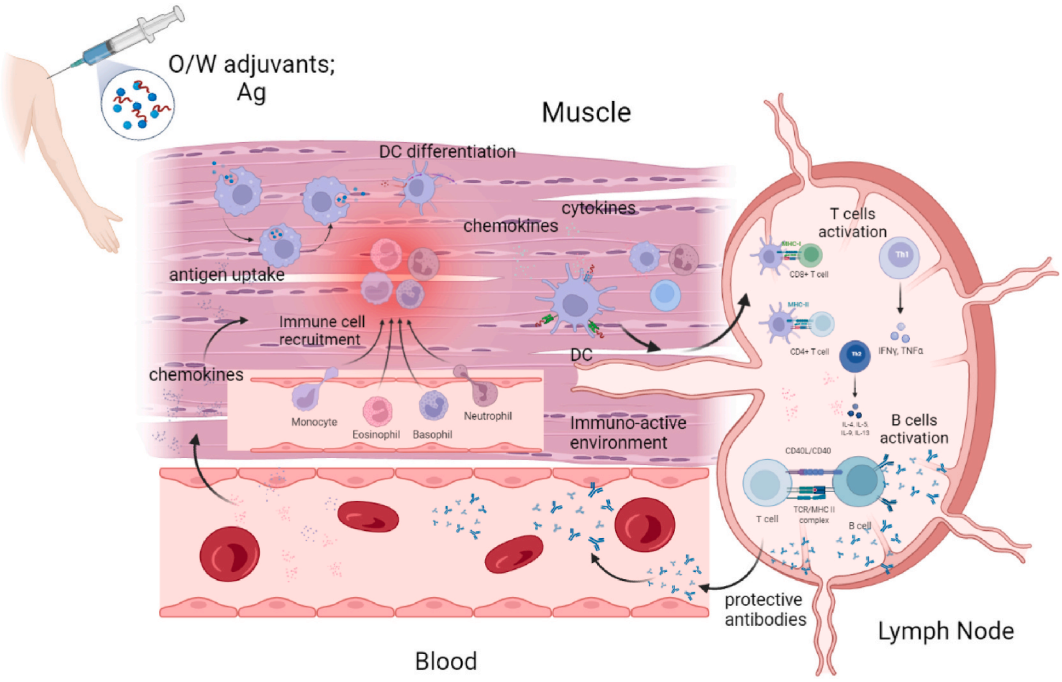

Fig. 2. The probable mechanism of action of oil-in-water emulsions. Following intramuscular injection, the oil-in-water emulsion creates an immunologically active environment at the injection site. Innate immune cells such as monocytes, dendritic cells, eosinophils, and neutrophils are recruited in large numbers in response to chemokines and subsequently recruited to the draining lymph nodes. Within the draining lymph nodes, DCs containing antigenic signals activate T and B lymphocytes, resulting in the production of Th1 and Th2 type cytokines and specific antibodies. The specific cytokines and antibodies then enter the bloodstream to produce a protective effect.

2.2. Oil-in-water adjuvants

Oil-in-water emulsions, with their unique shape structure, adsorb antigens onto the emulsion surface via electrostatic adsorption and chimaerism [28]. In addition to the unique mechanism of action of each adjuvant, the overall mechanism of action of oil-in-water emulsions is broadly based on the following procedure. Following intramuscular injection, an oil-in-water emulsion induces the recruitment of inflammatory cells, such as monocytes, dendritic cells (DCs), eosinophils, and neutrophils, to the injection site and their subsequent recruitment to the draining lymph nodes. Chemokines mainly induce immune cell recruitment [14,29]. In draining lymph nodes, antigen-loaded dendritic cells activate T cells, resulting in Th1 and Th2 phenotypes and the secretion of cytokines such as IFN-γ, TNF-α, IL-4 and IL-13. B-lymphocytes activated by dendritic cells differentiate into plasma cells and secrete antibodies. The antibodies enter the bloodstream and are transported throughout the body [30] (Fig. 2).

2.2.1. MF59

MF59 is the second human vaccine adjuvant approved by the U.S. Food and Drug Administration (FDA) after the aluminium adjuvant developed by Novartis. MF59 has been widely used as an adjuvant in clinical trials of various subunit vaccines such as influenza, hepatitis B, hepatitis C, and HIV vaccines [31]. Commercial formulations include squalene (oil phase), citric acid (water phase), Tween80, and Span85 (surfactant) [32]. Squalene is a direct precursor of cholesterol or steroid hormones synthesised in the liver, and is easily degraded and metabolised in the body [33]. From a production standpoint, squalene is available in large quantities and outperforms a range of vegetable oils in terms of purity, consistency, safety, and efficacy [34].

Furthermore, because squalene is insoluble in water [35] (water solubility of 0.124 mg/L), the coalescence of emulsified droplets due to Ostwald ripening should be minimal [36]. MF59 was prepared by using a microfluidic technique. The average particle size of the emulsion droplets produced by this method is approximately 160 nm and it can be stored stably for at least three years [37,38].

The following mechanisms of action of MF59 have been demonstrated in current studies: Unlike W/O Freund's adjuvant, MF59 does not form an antigen pool at the injection site. Instead, the antigens and adjuvants are gradually cleared at different kinetic rates [39]. MF59 stimulates antigen presentation at the injection site. MF59 directly enhances phagocytosis and cytosolic drinking, and promotes antigen presentation by antigen-presenting cells (APC) [40]. MF59 can mobilize immune cells to the injection site, and the main cell type that MF59 acting mainly on monocytes [9], which can be recruited to the site of adjuvant action, absorb antigens, and participate in the adjuvant-induced differentiation of the dendritic cell phenotype [41]. MF59 recruits APC for antigen presentation and transport to draining lymph nodes [42] and promotes antigen retention in lymph nodes and follicular dendritic cells [43].

The MF59 adjuvant vaccine is more likely to induce a Th2 immune-biased response [44,45]. Addition of the TLR9 agonist CpG or the TLR4 agonist E6020 to the MF59 adjuvant vaccine induces a more effective Th1 cell immune response, which is characterised by a higher IgG2a titre, enhanced interferon-gamma response, and similar or higher antibody titres [30,46]. HIV vaccines containing MF59 and Carbopol-971P (synthetic polyanionic carbomers) have been shown to enhance binding and neutralising antibody titres and have higher affinity [47,48]. Infants vaccinated with MF59-adjuvanted trivalent inactivated influenza vaccine (TIV) exhibit higher antibody titers and production of multifunctional cytokine $CD4^+$ T cells than children vaccinated with non-adjuvanted TIV [49,50].

Tests have shown the relative safety and reliability of MF59 vaccine adjuvant with no significant toxic effects in animal models or in human phase I-III clinical trials [51]. However, trials have also shown that the MF59 adjuvant can cause systemic reactions, including fever, headache, nausea, inflammation, and other symptoms. Another concern regarding the use of MF59 adjuvant is the relationship between oil-water emulsions and autoimmune diseases. Animal studies have shown that oil-water emulsions can cause autoimmune diseases such as autoimmune arthritis and autoimmune hepatitis, in susceptible subjects. However, further research is needed to determine whether humans and animal models have similar susceptibilities to oil-water emulsions [52,53].

2.2.2. AS02

AS02 is composed of AS03 components and two strong immunostimulants, QS-21 and MPL, which enhance its immunogenicity [13]. Inoculation with the MF59 adjuvant resulted in a Th2-biased immune response and a weak induction of the Th1 response in vivo [45]. The presence of QS-21 and MPL in AS02 not only induces strong humoral immune responses, but also activates and enhances the activity of T cells, facilitating their recognition and elimination of infected cells, thereby enhancing the breadth and depth of immune responses [54]. AS02 adjuvant vaccines have been used in studies of malaria, tuberculosis, hepatitis B, and HIV vaccines, tumour immunotherapy, and other studies [54]. In addition, intramuscular injection is more advantageous than subcutaneous injection in enhancing the immunogenicity and safety of the RSV-F vaccine combined with AS02 adjuvant after enhanced immunisation compared with MF59 adjuvant [55].

2.2.3. AS03

AS03 is a W/O adjuvant emulsion developed by GlaxoSmithKline Biologics with an average particle size of approximately 160 nm. In the commercial formula, the oil phase is squalene and α -tocopherol, the water phase is PBS buffer, and the surfactant is polysorbitol 80 (Tween80) [56]. Alpha-tocopherol is the main component of AS03, distinguishing it from other O/W emulsions [14]. Alpha-tocopherol is a type of vitamin E widely distributed in natureand is an immune enhancer that can be easily absorbed in tissues [57]. Another component of the AS03 adjuvant is the surfactant Tween 80, whose role is to stabilize the emulsion [58,59].

The AS03 adjuvant induces the production of cytokines and chemokines in the injection site muscle and draining lymph nodes by increasing NF-kB transcript levels. It can also activate and recruit monocytes and macrophages to increase antigen uptake. In addition, it recruits monocytes, dendritic cells, and granulocytes into the draining lymph nodes and increases the ability of APC to present antigens to $CD4^+$ T cells, which produce higher titres of antigen-specific antibody immune responses to B cells [56,60]. Like MF59,

AS03 generally induces Th2-biased immune responses and weakly affects Th1 responses [61]. Compared with MF59 adjuvant, α-tocopherol in AS03 adjuvant increases the level of induced cytokines, promotes the antigen uptake level of monocytes, and further increases the level of induced antibodies [56].

AS03 was first used in humans in a malaria vaccine [62]. More recently, this adjuvant has been used in human vaccines, particularly influenza vaccines. Clinical trials have shown that O/W adjuvants, such as AS03, induce a robust immune response when administered with influenza vaccines [63]. AS03 has been used in the HlN1 influenza virus vaccine (Pandemrix™), which induces a stronger humoral immune response than unadjuvanted vaccines [56]. AS03 has also been used in the H5N1 [64] and H7N9 influenza virus vaccines [65] in clinical trials. However, Pandemrix vaccination may increase the risk of narcolepsy [66]. Canadian Medicago and GlaxoSmithKline used the AS03 adjuvant in combination with a coronavirus-like particle (CoVLP) to prepare the CoVLP + AS03 vaccine (Covifenz, Medicago, Quebec City, Canada), which was the world's first approved plant-derived human vaccine. Canada was the first country to authorise the use of a plant-based COVID-19 vaccine [67]. Covifenz induces strong and long-lasting neutralising antibody levels and a balanced T cell response (IFN-γ and IL 4), both of which may help protect vaccinated individuals against COVID-19 [68]. Based on phase I and II clinical trial data, the level of neutralising antibodies produced by the Covifenz vaccine after vaccination is approximately 10 times higher than the serum antibody level of recovered COVID-19 patients, and the immune response of the cells can be detected in almost all vaccinated persons [69,70]. Phase III clinical trials showed that Covifenz was 69.5 % effective in the treatment of symptomatic volunteers infected with COVID-19, and it also had an effective preventive effect against COVID-19 variant strains [68]. Although the incidence of side effects after Covifenz vaccination is high, the vast majority of cases are mild to moderate [68–70].

As with other O/W adjuvants, the AS03 adjuvant causes systemic symptoms such as fever, headache, nausea, diarrhoea, and joint pain, which are associated with its induced inflammatory response [71]. However, the exact mechanism underlying drowsiness remains unknown. The inflammatory response induced by AS03 may also disrupt the body's autoimmune tolerance, and the induction of IL-17 may be a major cause of AS03-induced autoimmune diseases [72].

2.2.4. AF03

AF03 is an O/W vaccine adjuvant developed by Sanofi Pasteur. Its commercial formulation has a squalene oil phase, a PBS aqueous phase, and surfactants polyoxyethylene cetyl ether and Span85, with a Dv50 of approximately 90 nm [73]. Unlike the MF59 vaccine marketed by Novartis Vaccine Diagnostics and the AS03 influenza vaccine marketed by GlaxoSmithKline Biologicals, where the emulsion is prepared from squalene in water by micro-fluidization, AF03 is produced on an industrial scale through a phase in transition temperature (PIT) emulsification process [36,74].

The AF03 adjuvant was first used in the H5N1 influenza vaccine, and although the Humenza™ vaccine was never marketed, hundreds of volunteers were recruited for clinical trials. The AF03-adjuvanted vaccine demonstrated significant specific immune protection compared with the adjuvant-free vaccine, provided that it contained fewer antigens [75]. In addition, vaccination with the AF03 adjuvant significantly reduced the incidence and severity of interstitial pneumonia and prevented pulmonary and upper respiratory tract infections [76]. Although systemic adverse reactions did not differ significantly from those of non-adjuvanted vaccines, AF03-adjuvanted vaccines caused local adverse reactions more frequently than non-adjuvanted vaccines [75]. Recently, AF03 has been tested in Phase I/II clinical trials to develop a SARS-CoV-2 vaccine [77]. Manufactured by a unique method described in the article, AF03 also constitutes a delivery system for another emulsion adjuvant, AF04, a formulation containing a synthetic molecule (E6020) that purportedly mimics the properties of the natural product monophosphoryl lipid A (MPL) [78]. As previously observed in a glucopyrano-based lipid adjuvant (GLA)-stabilised emulsion (SE), the TLR4 agonist E6020 acted synergistically with the emulsion to enhance antibody and cellular immune responses in mice [78,79]. GLA and second-generation lipid adjuvants (SLAs) are substitutes for synthetic MPL-like molecules and TLR4 agonists. GLA and SLA adjuvants in emulsion formulations (SE) are being evaluated in clinical trials of vaccines against tuberculosis, schistosomiasis, leishmaniasis, and leprosy; however, they have also been evaluated in H5 influenza vaccines [80,81].

The use of emulsion adjuvants in the formulation of pandemic influenza vaccines has resulted in significant savings in the dose of haemagglutinin (HA) antigen, which facilitates the broad coverage of influenza vaccines. Although the frequency of injection site reactions to emulsion-adjuvanted influenza vaccines is generally higher than that of non-adjuvanted vaccines, these reactions are mild and limited to the first 2–3 days after injection [75].

2.2.5. Pickering emulsion

The Pickering emulsion used as a vaccine adjuvant is a microgel (alum) stabilised aqueous squalene emulsion. Its major difference fromtraditional surfactant emulsions is that the system does not contain a surfactant but uses nano-particles with certain hydrophilic and lipophilic properties as an emulsion stabiliser. By adjusting the particle properties and oil-water phase parameters, emulsions stabilised by particles were obtained by ultrasonic treatment [82]. Pickering emulsions have the advantages of being surfactant-free, less toxic, and more stable. They have a wide range of applications in the food, pharmaceutical, and cosmetic industries [32,83].

Some studies have shown that the Pickering emulsion is promising as a SARS-CoV-2 vaccine adjuvant to enhance humoral immunity against the novel coronavirus [84,85]. Alum adjuvants adhere more to the cell membrane than to dendritic cell (DC), resulting in a lack of intracellular transfer and antigen processing, thus limiting the Th1 cell-mediated cellular immune response [18,86,87]. To address this problem, alum is adsorbed onto oil-phase squalene using water as the continuous phase, resulting in an alum-stabilised Pickering Emulsion (PAPE). Owing to its main components, alum and squalene, PAPE has a good biosafety profile.

Furthermore, with the dense arrangement of alum at the oil/water interface, PAPE not only adsorbs large amounts of SARS-CoV-2 antigen, but the Pickering emulsion also has a higher affinity for DCs, thus triggering uptake and cross-presentation of the delivered

antigen [88]. As T-cell activation is determined by the amount of antigen present, APCs can adsorb and present more antigens, and the more T-cells are activated, the more pronounced the specific cellular immune response [89,90]. In the absence of a surfactant, the micron-sized particles and rough surface morphology of Pickering emulsions provide a large specific surface area for antigen adsorption and cellular interactions, allowing more effective antigen accumulation [91]. In addition, the PAPE group induced more than 6-fold higher titres of antigen-specific antibodies and 3-fold more IFN-γ-secreting T cells than the aluminium adjuvant group, indicating effective activation of humoral and cellular immunity [18].

Similar to the MF59 adjuvant, the adjuvant effect of the Pickering emulsion is due to the O/W emulsion formulation, and none of the individual components induce adjuvant-like effects [92]. Owing to the presence of alum particles, the Pickering emulsion forms an antigen pool at the injection site for approximately three days after injection, which then attracts DC cell infiltration and antigen uptake. Based on the available literature, the safety of Pickering emulsion as an adjuvant was assessed by immunopathology in serum and vaccine-related tissue damage; no significant side effects were detected at the injection site for several days after administration [93,94], and no cytokine storm production was detected [95]. However, these evaluations are only preliminary tests demonstrating the adjuvant properties and safety of PAPE. Although the side effects associated with the alum dose in Pickering emulsions are currently limited, whether the clinical neurotoxicity of aluminium will affect the widespread use of PAPE requires further study [18].

2.2.6. Syntex Adjuvant formulation (SAF)

SAF is an oil-in-water emulsion with squalane as the oil phase, developed by Syntex in the 1980s [38] to reduce the toxicity of CFA (W/O) emulsions with mineral oil as the oil phase, while still inducing an effective cell-mediated immune response. In PBS (pH 7.4), the final SAF composition before the addition of muramyl dipeptide was 5 % w/v squalane, 2.5 % w/v Pluronic L121, and 0.2 % w/v Tween 80 [96]. The final concentration of these components for injection is obtained after mixing with the antigen and/or muramyl dipeptide and diluting the stock solution by a factor of two [97,98]. The average particle size of SAF is 150–160 nm [98,99]. SAF emulsions (before the addition of cell wall acyl dipeptides) are very stable and can be stored at room temperature for six years without destruction, even under freezing conditions [96]. SAF emulsion stimulates the Th1 cell-mediated immune response, mainly manifested by increased IgG2a antibody level and increased secretion of cytokine IFN-γ [38,100]. SAF also activates the alternative complement pathway as another possible auxiliary mechanism of action [100]. Many different antigens are used in combination with SAF and exhibit good immune activity [38]. SAF has little muscle stimulation effect on the human body [96], but has shown high reactivity in clinical trials; therefore, it is no longer used as an immune adjuvant product, which may be related to the addition of muramyl dipeptides [38,101,102].

2.2.7. DETOX

Components of DETOX include bacterial cell wall skeleton (CWS) and monophosphoryl lipid A (MPL) in squalane (1 %) and Twain 80 (0.2 %) preparations [103,104]. DETOX, also known as Melacine, has good adjuvant activity and has been approved as a therapeutic vaccine for melanoma [105,106]. However, the side effects of DETOX adjuvants are obvious; they are reactive at the injection site and can lead to the formation of local granulomas [103,107].

2.2.8. CoVaccine HT

The CoVaccine HT adjuvant is an oil-in-water emulsion composed of 8 % w/v squalane, 2 % w/v Tween 80, and 2 % w/v immunostimulant (sucrose fatty acid sulphate ester) in PBS. It has been found to be effective in inducing humoral and cellular responses in pigs [105,108,109]. However, the CoVaccine HT antihypertensive vaccine was terminated in 2010, after serious dose-related adverse reactions occurred in a Phase II clinical trial in 2008 [110,111]. Recent studies have shown that the recombinant protein subunit SARS-CoV-2 vaccine formulated with the CoVaccine HT adjuvant can induce a broad-spectrum IgG response and high-titre neutralising antibodies (NtAbs) against the original and mutated strains of SARS-CoV-2 in mice. It also produces an antigen-specific IFN-γ secretion response in distant mice [112].

2.2.9. PROVAX

PROVAX is a squalane emulsion developed by IDEC Pharmaceuticals, which is formulated similarly to SAF, but reduces the concentration of Pluronic L121 to 1.25 % w/v when injected and removes muramyl dipeptides [104]. PROVAX is a triple concentrate composed of 15 % w/v squalane, 3.75 % w/v Pluronic L121, and 0.6 % w/v Tween 80, which can induce antibodies and cytotoxic T cells [38,113].

2.2.10. Stable emulsion (SE)

SE is an emulsion adjuvant originally developed by Edgar Ribi and subsequently developed by the US Institute of Infectious Diseases. It is similar to MF59 but contains lower squalene content, a phospholipid emulsifier, and a lower concentration of alpha-tocopherol (0.01%v/v) [104,114]. The specific formula of SE is 10 % v/v squalene, 1.9 % w/v lecithin, 0.091 % w/v Pluronic F68, 0.05 % w/v α-tocopherol, and 1.8 % v/v glycerol in 25 mM ammonium phosphate buffer (pH 5.1) [115,116]. The addition of MPL to SE can produce MPL-SE, a potent adjuvant that, as an adjuvant for leishmaniasis vaccine, exhibits Ag-specific Th1 immune responses characterised by powerful IFN-γ production upon specific Ag re-exposure in vitro [117]. This is similar to the effect of MF59 in inducing a mixed Th1/Th2 cell response [61]. SE was evaluated in clinical studies of pandemic H1N1 influenza but was never licenced. However, the Infectious Disease Research Institute (IDRI) continues to develop SE and other adjuvant emulsions [4,117,118]. The manufacturing process of SE has successfully moved to other locations to facilitate local vaccine production [114], and manufacturing innovation continues with a view to eventually produce a pandemic vaccine globally.

3. Discussion and conclusion

Emulsion-adjuvant vaccines are safe, and their efficacy and immune effects are better than those of non-adjuvant vaccines for influenza [12,14,15]. They can save antigen doses and be mass-produced to cope with growing demand; in addition, emulsion vaccines respond to homologous and heterologous strains through a cross-immune response [61]. Given the characteristics of emulsion adjuvants, their inclusion in protein-based vaccines is currently being investigated as a medium-to long-term response to the SARS-CoV-2 pandemic [119,120]. The combination of emulsion adjuvants and subunit vaccines can meet the needs of the pandemic response because they can significantly reduce the antigen content, save vaccine doses, and be rapidly manufactured. Emulsion adjuvants can bind to recombinant proteins, engineered nanoparticles, and whole inactivated viruses. However, their effects on antigenic integrity need monitoring on a case-by-case basis [61]. SARS-CoV-2 vaccines based on MF59 and AS03 adjuvants are currently in an advanced stage of development [69,120]. Therefore, emulsion-adjuvant SARS-CoV-2 vaccines are expected to play an important role in the global pandemic.

In the context of the ongoing SARS-CoV-2 pandemic, where viral mutations and antigenic transfer are prominent, the potential of emulsion adjuvants to induce a broad, potentially cross-protective immune response could provide considerable coping advantages. Although current preliminary data are encouraging [121], careful evaluation of the immune response induced by emulsion adjuvant vaccines relative to alternative vaccine approaches is needed. The design of optimal antigen structures using bioinformatics methods and protein engineering is also critical for successful vaccine preparation [120,122]. Decades of experience with the application of emulsion adjuvants, including their critical role in responding to an influenza pandemic, have provided a solid foundation for the establishment of an emulsified adjuvant vaccine platform. Owing to their long history of safe use in humans and known modes of action, emulsion adjuvants represent a benchmark for the development of novel vaccine adjuvants. Emulsion adjuvants are the adjuvants of choice for treating pandemic diseases, including those caused by influenza and other emerging pathogens.

Research on adjuvants, especially promising emulsion-based adjuvants, is essential for the development of modern subunit and peptide vaccines. The fact that MF59 is the second adjuvant to be successfully marketed after the aluminium adjuvant, and that AS03 emulsion-based adjuvants have already been marketed for use in influenza vaccines shows that emulsion-based adjuvants have a promising future for the development of vaccine adjuvants. In addition, an in-depth study of the molecular mechanism of action of emulsion-based adjuvants in inducing protective immune responses in hosts will open new pathways for the design and improvement of vaccine adjuvants, which will help accelerate the development of vaccines against novel coronaviruses and even chronic infectious diseases such as AIDS, hepatitis C, and tumours.

The use of different adjuvants in combination with the same vaccine to enhance immune response may be a promising direction for future research. Recently, measures such as mixing and matching vaccinations, developing new vaccines such as nanoparticle vaccines, and optimising immune adjuvants are expected to improve vaccine safety and efficacy [123]. For example, co-loading the TLR4 agonist MPLA and the TLR9 agonist CpG into synthetic HDL nanodiscs resulted in an adjuvant system (ND-MPLA/CpG) that significantly enhanced dendritic cell activation compared with the non-adjuvanted group, and mixing with the OVA antigen significantly enhanced humoral immunity in mice [124]. This suggests that mixing adjuvants with different mechanisms of action can enhance the immune response through different immune pathways or different parts of the immune response, and can even alter the type of immune response.

Future studies on vaccines and adjuvants should adequately evaluate their safety and efficacy. They should be effective in inducing strong cellular and humoral immune responses and forming relevant memory cells, while simultaneously balancing the safety of vaccines and adjuvants, ideally with their availability and accessibility to special populations including pregnant women, the elderly, immunodeficient patients, transplant recipients, and cancer patients. Only in this way can the cause of human immunisation be taken to the next level, and even to the next milestone.

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Data availability statement

Data was included in article/referenced in article.

Ethics declarations

Review and/or approval by an ethics committee was not needed for this study because animal experiments or clinical studies are not required for this study.

CRediT authorship contribution statement

Zhuanqing Huang: Writing – original draft. **Hui Gong:** Writing – review & editing. **Qi Sun:** Writing – review & editing. **Jinjin Yang:** Supervision. **Xiaochuan Yan:** Supervision. **Fenghua Xu:** Funding acquisition.

Declaration of competing interest

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

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