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Vaccine Adjuvants: Selection Criteria, Mechanism of Action Associated with Immune Responses and Future Directions

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ABSTRACT

The most effective method to minimize the prevalence of infectious diseases is vaccination. Vaccines enhance immunity and provide protection against different kinds of infections. Subunit vaccines are safe and less toxic, but due to their lower immunogenicity, they need adjuvants to boost the immune system. Adjuvants are small particles/molecules integrated into a vaccine to enhance the immunogenic feedback of antigens. They play a significant role to enhance the potency and efficiency of vaccines. There are several types of adjuvants with different mechanisms of action; therefore, improved knowledge of their immunogenicity will help develop a new generation of adjuvants. Many trials have been designed using different kinds of vaccine adjuvants to examine their safety and efficacy, but in practice, only a few have entered in animal and human clinical trials. However, for the development of safe and effective vaccines, it is important to have adequate knowledge of the side effects and toxicity of different adjuvants. The current review discussed the adjuvants which are available for producing modern vaccines as well as some new classes of adjuvants in clinical trials.

Keywords: Adjuvants, Immune system, Immunogenic feedback, Licensed adjuvants, Vaccine

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INTRODUCTION

The most effective and potential way to combat different types of infectious diseases is vaccination. Vaccination aims to provide long-term protection and induce pathogen-specific immune feedback [1]. Conventional vaccination methods formulated with inactive or live attenuated pathogens can induce long-term and potential stimulation of the immune mechanism, but these vaccines are correlated with many health problems such as potential mutations that can re-establish pathogenicity or partial inactivation of antigens. Similarly, subunit vaccines are inoffensive but are less effective. So, to increase the immunogenicity of subunit vaccines, adjuvants are essential [2]. Adjuvants are usually defined as biochemical substances which are added to the composition of the vaccines to enhance the immune response against any pathogens [1]. They activate immunization properties using small doses of the vaccine with some amount of antigen in the associated vaccine [3]. Moreover, adjuvants assist to boost the consistency of vaccines by making them less vulnerable to the demolition that would happen upon injection or storage. They also stimulate the onset, stability, and the duration of the immune feedback [4]. Adjuvants should be used when required for the development and controlling determination, vaccine composition should be kept as simple as possible, adjuvants can be important for antigen/dose-prudent, enlargement of immunity against mutable antigens, and increasing feedback from susceptible populations with ineffective immune feedback. The description of the adjuvant mechanism of action (MOA) is significant for active translation and progressive anticipation from a monitoring and licensing perspective. During the depot effect, adjuvants entangle, engross, or assemblage antigens and deliver them steadily over a prolonged period [5]. At the place of injection, such a depot effect also restricts the dropping of antigens by liver evacuation [6]. With the help of the depot effect liposomes take action [7]. At the place of injection, adjuvants evoke regional pro-inflammatory immune feedback inducing to recruitment and stimulation of immune cells. Redness, swelling, and pain may also result from local inflammation [8]. At the site of injection, recruiting of immune cells activates MF59 [9]. By altering the cytokine complex immunomodulatory adjuvants induce the immune system. Initially, enhancing the concentrations of some cytokines and reducing the concentrations of other adjuvants influence the type of immunity. Th1 feedback and cell-mediated immunity are associated with IL-2, IL-12, and Interferon-gamma (IFN-γ), therefore Th2 feedback and humoral immunity are associated with IL-4, IL-5, IL-6, IL-13, and probably IL-10 [10]. By stimulating Th1 feedback Monophosphoryl lipid (MPL) takes action [11].

To increase humoral immunity, aluminum salts (alum) have been commonly used, but their MOA is still under observation [12]. Over the past 2 decades, better consideration of innate immunity including the understanding of pattern recognition receptors (PRRs) has updated the development of novel adjuvants [13]. Adjuvant immunostimulatory actions can regulate unwanted reactogenicity, so during the developmental process, it should be considered that vaccine adjuvants not only be immunogenic but also extremely tolerable. These review objectives are to introduce the discovery of adjuvants, the criteria to be adjuvants, classification and the current mechanism of action, licensed adjuvants, their role in innate and adaptive immune responses, obstacles in the development, and the future directions.

Adjuvant's Discovery

The adjuvants discovery was observed as an emerging technique to plan vaccines against deadly pathogens such as HBV and HIV. Due to the lack of knowledge of their mode of action, based on existing experiments, molecules were preferred to develop with different antigens. Among the diversity of adjuvant molecules, those that develop more

immunogenicity due to their characterization and promising effects were selected. In 1893, the first adjuvant consideration was reported that supervision of dead bacteria (Coley toxins) may be valuable in the treatment of some kinds of cancer [14]. In 1925, Ramon detected at the site of injection that some ingredients influencing sterile inflammation which were capable of enhancing antisera production of diphtheria and tetanus [13]. In 1926, Glenny observed that alum increased antibody feedback; alum was commonly used in different human vaccines as an adjuvant. In 1960, water-in-oil emulsions were inhibited due to their higher side effects, and they were rapidly observed by the production of oil-in-water emulsions. In 1970, antigen-encapsulated or adsorbed liposomes and virosomes were developed. In the early 1980s, MPL and its different formulations were discovered, QS-21 was recognized in the late 1980s, and CpG was recognized in 1995. As they were determined to stimulate different features of the adaptive immune feedback, such molecules were called immune-modulating molecules. In 1990, the hepatitis vaccine that used virosomes as an adjuvant was licensed, and the first non-live vaccine was produced [15]. However, the adjuvant's mechanism of action and their characterization were padded for several years [16]. The discovery of PRRs and their antagonist in the 1990s and early 2000s revealed different new prospects in adjuvant development and discovery. Many adjuvants to activate different kinds of innate immune feedback can trigger PRRs indirectly or directly and can induce and increase specific parts of the adaptive immune system if combined with an antigen [17]. The US Food and Drug Administration (FDA) 2009 accepted the first unique adjuvant-based vaccine which comprises adjuvant system (AS)04-involved alum and TLR4A agonist Monophosphoryl lipid A (MPLA), against human papillomavirus. Some FDA-approved adjuvants used in the human vaccine are mentioned in Table 1. Afterward, globally different vaccines were approved with unique adjuvants, such as HBV containing TLR9 ligand CpG oligodeoxynucleotide (CpG-ODN) or AS04 and the subunit zoster vaccine with AS01B.

Table 1. FDA-approved adjuvants used in human vaccines

Adjuvants	Vaccine product	STN/Patent number
Amorphous aluminum hydroxyphosphate sulfate	Hepatitis B Vaccine	BL 101066/5768
MF59	Influenza Vaccine, Adjuvanted	BL 125510/236
Aluminium hydroxide	Anthrax Vaccine Adsorbed	BL 103821/5344
Aluminum phosphate	Diphtheria & Tetanus Toxoids Adsorbed	BL 103944/5183
Aluminum hydroxide	Hepatitis A Vaccine, Inactivated	BL 103475/5689
Amorphous aluminum hydroxyphosphate sulfate	Human Papillomavirus 9-valent Vaccine, Recombinant	BL 125508/787
Aluminum hydroxide	Japanese Encephalitis Virus Vaccine, Inactivated, Adsorbed	BL 125280/251
Aluminum phosphate	Meningococcal Group B Vaccine	BL 1225549/737
Aluminum phosphate	Tetanus Toxoid, Reduced Diphtheria Toxoid and Acellular Pertussis Vaccine, Adsorbed	BL 125111/814
Amorphous aluminum hydroxyphosphate sulfate	Haemophilus b Conjugate Vaccine	BL 103237/5285
AS01B	Zoster Vaccine Recombinant, Adjuvanted	BL 125614/398
Aluminum hydroxide	Menactra Meningococcal Group B Vaccine	BL 125546/824
Aluminum hydroxide	Diphtheria & Tetanus Toxoids & Acellular Pertussis Vaccine Adsorbed	BL 103647/5552
Aluminum phosphate	Tetanus & Diphtheria Toxoids Adsorbed for Adult Use	BL 103171/5221

By incorporating the different molecules (MPL, QS-21, CpG) with traditional adjuvants (aluminum, liposomes, oil-in-water emulsions) to develop the adjuvant system, it was proposed to derive, at the minimum, a supplement consequence on the adaptive immune feedback. Therefore, it was assumed that a harmonious or even coactive effect on the subsequent responses (cellular as well as humoral components) would take place, and possibly an influence on the magnitude of the immune feedback would be examined. Compared with the non-adjuvant or alum-based adjuvant characterization, the adjuvanted vaccine had to activate powerful effective immune feedback against the antigen, manifest a tolerable reactogenicity configuration in the designed population, and be physically adaptable with the antigen. It was speedily recognized that the molecular domain (i.e., the vaccine development) was as crucial as the molecule by its nature in the formation of an effective and feasible adjuvant system [18].

Criteria for a Substance to be Adjuvant

It should:

- be chemically pure and have a defined configuration
- not provoke autoimmunity
- potentially activate the vaccine
- activate a strong humoral and T cell immune feedback
- be suitable for cataloging as a supplementary vaccination
- deliver better immunological memory or long-term immunity
- remain constant under a wide-range of time, storage, temperature, and pH
- be bioabsorbable, biodegradable, safe, sterile, low-priced, and easy to handle and store.

It is well recognized that if subunit vaccines are combined with an adjuvant, they provoke more effective and resilient antigen-specific immunity. Two principal components can be used for in vivo adjuvant effects such as the delivery system and immune potentiation [19, 20]. Delivery systems restrict antigens and target them to suitable cells of the innate immune mechanism. Delivery can also be enhanced for immune potentiator targeting. Immune potentiation provides the pro-inflammatory environment for antigen recognition by activating directly innate immune cells. The particular pathogens' epitopes provided by the antigens are compulsory to develop prolonged immunological memory. For naturally occurring infections and whole-cell vaccines, these components are intrinsic, while they must be utilized in subunit vaccine composition [21]. Some common examples of adjuvants are mentioned in Table 2.

Adjuvant's Classification

Classification of the adjuvants based on the source, physiochemical properties, and their mechanism of action. Adjuvants are classified into three main groups as described by Edelman [22]:

1. Active immune stimulants are the elements that enhance the immune feedback to the antigen such as flagellin, saponins (QS-21), and muramyl dipeptide (MDP).
2. Carriers, as immunogenic proteins that induce T cells activities such as virosomes, calcium phosphate, and cochleate.
3. Vehicle adjuvants, liposomes, or oil emulsions assist the antigen for matrix, and activate the immune feedback.

Adjuvants: The General Mechanism of Action

Adjuvants imitate pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs) from pathogens that are predicted by the innate immune mechanism (Figure 1). This activates the local cytokine feedback and the mobilization of different innate cells including immature DCs and monocytes. Immature innate cells begin to mature in APCs after the integration with pro-inflammatory signals. Simultaneously, they induce T and B cell feedback after migrating toward their local region in the draining lymph nodes. Such a

Table 2. Some common examples of vaccine adjuvants

Category	Example
Mineral salts	Aluminium hydroxide
	Aluminium phosphate
	Calcium phosphate
	Cytokines e.g., IL-2, IL-12, GM-CSF
	Saponins e.g., QS21
	MDP Derivative
	CpGoligos
	LPS
	MPL
	Polyphosphazenes
Lipid particles	Emulsions e.g., FCA, SAF, MF59
	Liposome's
	Virosomes
	ISCOMS
	Cochleates
Micro particulate adjuvants	PLG micro particles
	Poloxamer particles
	Virus-like particles

Adapted from Sailaja AK et al, [60]. Interleukin (IL)-2, Interleukin (IL)-12, Granulocyte-macrophage colony-stimulating factor (GM-CSF), Muramyl dipeptide (MDP), Monophosphoryl lipid A (MPL), Lipopolysaccharides (LPS), Freund's Complete Adjuvant (FCA), Syntex adjuvant formulation (SAF), immune stimulating complex (ISCOM), poly-lactide-co-glycolide (PLG)

mechanism further influences the production of adaptive immune effectors like antibodies and $CD4^+$ T cells. Therefore, adjuvants after their possible effects on the innate response can stimulate the quality and proportions of the adaptive immune response. Moreover, adjuvants can influence the immune profile of the adaptive immune system and may express a better quantity and quality of enhanced cytokine patterns, a broader profile, and a larger diversity of $CD4^+$ T cells. Some common adjuvants with their mechanism of action are described in Table 3.

Current Uses of Licensed Adjuvants

In the United States, alum is extensively used as an adjuvant and approved nationally [23]. More than 70 years ago, alum-based vaccines were licensed. In 1997, in the European market, an influenza vaccine adjuvanted with an alternative known as MF59 was effectively propelled [24]. Furthermore, AS04 has been accepted in Europe and certified in the United States, which is a combination adjuvant comprising MPL adsorbed to alum [23]. Among the non-small-cell lung cancer (NSCLC) vaccine, Montanide ISA51 is broadly used as an adjuvant, certified by Chile and Cuba [25]. Illustrations of licensed adjuvants with their properties are described in Table 4.

Significance of Adjuvants in Innate and Adaptive Immune Responses

If any foreign particle or antigen invades the body, the immune system immediately becomes active to give a response to them [26]. Sometimes the immune system responds speedily or maybe slowly. The first line of protection is the immediate feedback of the body's immune system while a gradual and long-term feedback is provided by the adaptive immune method [27]. During the innate immune feedback, both the complement and phagocytic cells play their role in defense against the antigens. Antigen-mediated activation of T cells and B lymphocytes initiates the adaptive immune feedback that has antigen-specific surface receptors. Two different types of T cells such as $CD4^+$

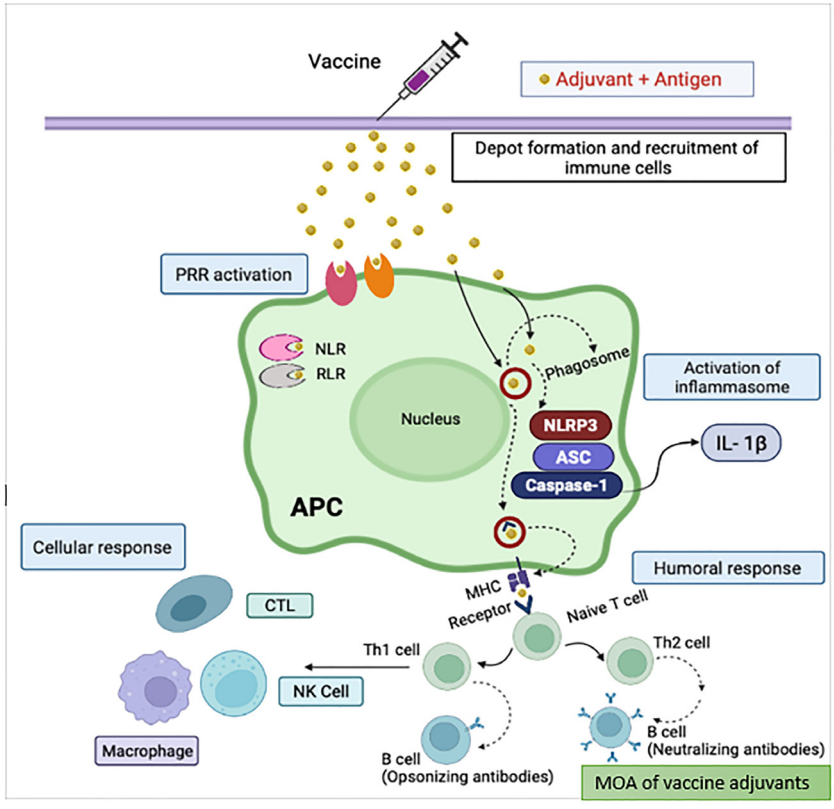

Figure 1. Adjuvants general mechanism of actions. At the site of injection adjuvants develop depot of antigens and organize immune cells. They can trigger PRRs of organized APCs before or during endocytosis of antigens, after that antigen progressed and confer to T cells evolving in humoral and cellular feedback. Apoptosis-associated speck-like protein containing a caspase recruitment domain (CARD) (ASC), LRR- and pyrin domain-containing protein 3 (NLRP3), Pattern recognition receptors (PRR), NOD-like receptor (NLR), RIG-I-like receptor (RLR), antigen presenting cell (APC), T helper type 1 (Th1), T helper type 2 (Th2), major histocompatibility complex (MHC), Mechanism of action (MOA), Cytotoxic T lymphocyte (CTL), Natural Killer (NK) cell

Th cells (Th) cells and $CD8^+$ cytotoxic T lymphocytes (CTLs) are involved in the body's immunity, while Th1 and Th2 cells are the subpopulations of further divisions of the Th cells which are very important [28]. PRRs expressed by the innate immune cells help to identify foreign infectious agents or antigens. PRRs also have different families such as retinoic acid-inducible gene-1 (RIG-1) like receptors (RLRs), C-type lectin-like receptors (CLRs), TLRs and nucleotide oligomerization domain (NOD) like receptors (NLRs). NLRs and RLRs are placed intracellularly while TLRs and CLRs are located on the APCs [4]. Pathogenic microorganisms such as bacteria, viruses, parasites, and fungi represent the PAMPs [29]. Before or during the endocytosis of an antigen, APCs can identify PAMPs via PRRs. Antigens undertake modifications in APCs after recognition and antigen peptides are transferred toward major histocompatibility complex (MHC) molecules. Afterward, the stimulation of cellular immunity and humoral immunity becomes functional with the activation of Th cells by the combination of MHC class II molecules complexed with the antigen peptides. However, the stimulation of $CD8^+$ cells initiates the cellular responses by the activation of MHC class I molecules complex with antigen peptides [30]. Commonly, adjuvants with the help of PRRs stimulate the innate immune system in immune cells. For direct increasing of an activation pathway by the secretion of cytokines activated by the help of adjuvants complex with PRRs, commonly immunostimulatory adjuvants act as ligands for PRRs. Receptor-ligand associations lead

Table 3. Some common adjuvants with their mechanism of action

Adjuvant	Mechanism of action	Ref.
Emulsion o/w	Innate inflammatory responses, activation and recruitment of APCs, enhancement of antigen persistence at the site of injection and delivery to immune-competent cells	[37]
Aluminium salts	Activation of innate immune responses	[37]
Emulsion w/o	Induction of local inflammation, activation and recruitment of APCs	[37]
Liposomes	Depot formation, presentation of antigens to APCs	[61]
Virosomes	Delivery of antigens to APCs	[62]
QS21	Presentation of antigens to APCs, induction of CTL production, stimulating both Th1 and Th2 cytokine secretion	[37]
ISCOMs	Induction of balanced Th1 and Th2 responses	[6]
PLGA	Cross-presentation of antigens to CD8? T cells	[32]
Chitosan	Translocation of tight junctions of cells	[32]
MPI	Stimulation of both Th1 and Th2 responses	[63]
IFN- gamma	Up-regulation of Th1 response	[34]
IL-1	Maturation of T and B cells	[34]
IL-2	Up-regulation of Th1 responses	[34]
IL-4	Up-regulation of Th2 responses	[34]
IL-12	Induction of strong Th1 shift	[34]
GM-CSF	Activation and recruitment of APCs	[64]
VLPs	Direct activation of B cells, stimulation of DCs, induction of cross-presentation to CD8+ T cells	[32]
CT	Stimulation of Th2 responses	[35]
CpG motifs	Stimulation of Th1 responses	[64]
MPL	Stimulation of Th1 responses	[34]
MDP-lipophilic	Stimulation of Th1 responses	[34]
MDP-hydrophilic	Stimulation of Th2 responses	[34]

Antigen presenting cell (APC), Cytotoxic T lymphocyte (CTL), T helper type 1 (Th1), T helper type 2 (Th2), immune stimulating complex (ISCOM), Poly lactic-co-glycolic acid (PLGA), Micro-particulate inulin (MPI), Interferon gamma (IFN-γ) , Interleukin (IL) Granulocyte-macrophage colony-stimulating factor (GM-CSF), virus-like particle (VLP), Cholera toxin (CT) Cytosine guanosine dinucleotide (CpG) , Monophosphoryl lipid A (MPL), Muramyl dipeptide (MDP) Dendritic cell (DC)

Table 4. Licensed adjuvants

Adjuvant	Vaccine	Ref.
AS03	Influenza (H5N1, H1N1)	[25]
As04	Hepatitis B, human papillomavirus	[32]
Thermo-reversible emulsion (o/w)	Influenza (H1N1)	[25]
Virosomes	Influenza, Hepatitis A	[37]
Alum	Hepatitis A, B, diphtheria/tetanus/pertussis (DTP)	[32]
MF59	Influenza (H1N1, H5N1, seasonal)	[25]

Adjuvant system 04 (AS04), Adjuvant system 03 (AS03)

to the manifestation of genes that encode the cytokines, chemokines, and co-stimulatory molecules which perform the function of expansion, priming, and the polarization of immune feedbacks. Consequently, due to inflammasome constituents of dying or injured host cells, they also take part in the functional activity of adjuvants [3]. In particular, for a diversity of immune responses, TLR ligands act as a potent immunomodulator. TLRs help in the recognition of different constituents of bacteria and viruses. TLR ligands also include different classes such as protein, lipid, and nucleic acid components [31].

Diverse patterns of gene expression are induced when different TLR ligands stimulate cells, representing variations in signaling pathways that arise through particular usage of toll/interleukin-1 receptor domain-containing adaptor protein inducing interferon-beta (TRIF) and adaptor molecules like myeloid differentiation primary response gene 88 (MyD88) among TLRs. Inflammatory cytokine production is stimulated by the activation of nuclear factor kappa B (NF-κB) through MyD88, while type-I interferon production is stimulated by the activation of the transcription factor interferon regulatory factor 3 (IRF3) through TRIF. Lipopolysaccharide is a well-recognized TLR ligand, triggering TLR4 [30]. Meanwhile, CpG oligodeoxynucleotides are accepted by TLR9, polyinosinic: polycytidylic acid (poly-I: C) stimulates TLR3, imidazoquinolines are accepted by TLR7/8 and flagellin is acknowledged by TLR5 [31]. Thus, adjuvants that have similar structures in association with different ligands of PRRs can trigger their corresponding receptors, resulting in the activation of innate immunity. In contrast, by increasing T cell responses, adjuvants can induce adaptive immune feedback. Immune-stimulating complexes (ISCOMs) act by triggering antibody production and stable Th1 and Th2 immune feedback [32], while MPL provokes Th1 feedback [33], and cholera toxin (CT) induces Th2 feedback [34]. Cell-mediated immunity is triggered by Th1, whereas humoral feedback is triggered by Th2 cells which neutralize the extracellular antigens. For the production of $CD8^+$ T cell responses through adjuvants, there are different challenges in the development of unique adjuvants. A favorable adjuvant should be merged with an antigen in such a fashion that enables admittance of the antigen into the MHC class I processing pathway, stimulating type-I IFN production and inducing dendritic cell (DC) activation to increase the differentiation of functional CD8 T cells [3]. A study performed by Bungener et al. illustrated that ovalbumin carried by fusion-active virosomes triggered class I MHC-restricted CTL feedback. They also reported the significance of virosomes as a model antigen delivery system that can stimulate cellular immunity against condensed protein antigens [35].

Obstacles in Adjuvant Development

At the injection site, adjuvanted vaccines show greater reactogenicity in contrast to non-adjuvanted vaccines [36]. Therefore, adjuvants not only enhance the immunogenic feedback of antigens but also provide serious side effects. Sterile abscess and granuloma formation along with local swelling at the booster site are serious side effects related to adjuvants. During laboratory experiments with animals, systemic reactions such as anterior uveitis, malaise, adjuvant arthritis, and fever are also observed [37]. Several candidates of adjuvants are available but a limited number of adjuvants among them are licensed and successfully utilized in the vaccine composition. Adjuvant failure mainly depends on safety issues. Mostly, temporary safety matters hamper the expansion of adjuvants. But long-term and intermediate safety issues are major challenges to reduce [38]. However, the directions of adjuvant production should not only detect highly provocative adjuvants but also should pay attention to unique approaches to minimize the effects of the reactogenicity of adjuvants [39]. Furthermore, there is a lack of in vitro assessment that can entirely initiate in vivo immune feedback, because of the heterogeneous characterization of the immune mechanism. Thus, different animal models are utilized for most preclinical experiments. Such experiments can provide some knowledge on the safety and effectiveness of vaccines, but may not promise that parallel results will be detected in husbandry target animals or humans [40]. Several adjuvants are under clinical investigation as shown in Table 5.

Advancements in the Adjuvant Formulation

The latest approach for adjuvant formation

Table 5. Adjuvants in different clinical phases under investigation

Type of adjuvant	Adjuvant name	Clinical phase	Condition	Ref.
Saponins	Matrix M	Phase 1	Malaria	[64]
		Phase 3	Respiratory F-protein	[65]
		Phase 2	Melanoma	[64]
Cytokines	GM-CSF	Phase 2	Hepatitis B	[64]
	IL-12	Phase 1	HIV	[66]
	IL-15	Phase 1	HIV	[66]
Lipids	GLA-SE	Phase 1	Malaria	[67]
	GLA-AF	Phase 1	Influenza	[68]
	CCS	Phase 2	Hepatitis B	[69]
	MPL	Phase 2	Influenza	[70]
Nucleotide	CpG 7909	Phase 1	Malaria	[71]
	Interleukin-2	Phase 1	HIV	[72]
	dsRNA	Phase 1	HIV	[73]
	IL-12 DNA	Phase 1	Influenza	[74]
Emulsions	Montanide ISA 51	Phase 1	Malaria	[75]
	Montanide ISA 720	Phase 2	Malaria	[75]

Granulocyte-macrophage colony-stimulating factor (GM-CSF), Interleukin (IL), Human immunodeficiency virus (HIV), glucopyranosyl lipid adjuvant–stable emulsion (GLA-SE), Aqueous formulation of glucopyranosyl lipid adjuvant (GLA-AF), Monophosphoryl lipid A (MPL), polycationic sphingolipid (CCS), Cytosine guanosine dinucleotide (CpG)

could be one that allocates significant attention to testing many developing adjuvant ideas in small clinical trials (phase 0/I), preliminary in their growing pipeline. Therefore, the systems' vaccinology techniques used to achieve mechanistic visions and novel adjuvants can be promptly evaluated in the small phase I (phase 0) human experiments. For instance, Sékaly and teammates investigated the innate immune feedback in humans to synthetic double-stranded RNA poly(I:C) balanced with poly-L-lysine stabilized in carboxylmethyl cellulose (poly-ICLC), an agonist for TLR3 and melanoma differentiation-associated protein 5 (MDA5) [41].

Furthermore, the systems' vaccinology approaches can deliver visions about the mechanism of actions of adjuvants in humans. These are advantageous for adjuvants that are presently used in licensed vaccines, including saponin-based adjuvants, such as AS01b as well as squalene-based adjuvants, such as AS03 and MF59, which do not appear to evoke the canonical TLR-subordinate or other PRR-subordinate pathways of innate stimulation. Concerning saponin-based adjuvants, Matrix-M1 another saponin-based adjuvant during the recent COVID-19 vaccine clinical trial expressed high proportions of neutralizing antibody titers (Table 6) [42] but there is an insufficient insight into the cellular and molecular mechanisms by which these kinds of saponin-based adjuvants negotiate their effects [43]. Moreover, the systems' vaccinology approaches can help in the mechanisms by which synthesis work, the basic mechanism of vaccination that produces adverse reactions soon after the injection, and for the vaccine delivery the rational design of optimal synthesis [44]. The outcomes carried from these phase 0/I trials will authorize the implementation of a mechanistic hypothesis about adjuvants and then can be examined in animal models or in vitro human organoid cultures [45].

Adjuvant's Safety

The essential part of vaccine formulation is the safety of the vaccine as it considers the potency, and immunogenicity. Vaccines are frequently formulated with vital ingredients (antigens) and other different molecules such

Table 6. Some common adjuvants used in Coronavirus vaccines

Adjuvant	Manufacturer	Vaccine	Status	Ref.
Alum	Sinopharm Sinovac	inactivated whole SARS-CoV2 virus vaccine	In certain countries approved for limited or emergency use	[76]
AS03-GSK	GSK (AS03) Sanofi (antigen) Medicago (antigen)	Recombinant S-protein expressed in Baculovirus Virus-like particles (CoVLP)	Phase I/II Phase III	[77]
Matrix-M	Novavax	Recombinant SARS-CoV-2 spike (S) protein	Phase III	[78]
MF59 - Seqirus	University of Queensland	Molecular clamp-stabilized S protein	Phase I testing on-going	[79]
CpG 1018	Dynavax (CpG 1018) Medicago (antigen)	Recombinant SARS-CoV-2 spike (S) protein on virus like particles	Phase I/II	[80]
TLR7/TLR8 ligand adsorbed in alum	Bharath Biotech	Inactivated SARS-CoV-2 vaccines	Phase III/emergency use in India	[81]

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), GlaxoSmithKline (GSK), Cytosine guanosine dinucleotide (CpG), Toll-like receptor (TLR)

as adjuvants, preservatives, stabilizers, and some other surplus ingredients used in the process of development. The evaluation of the safety confirmation of adjuvanted vaccines was observed on the final product injected into humans, rather than individual ingredients as for all vaccines. At all clinical and pre-clinical stages of formulation, vaccine safety is evaluated and perused after licensure to the entire life of the product. Those vaccines are developed with novel adjuvants; administrative authorities should observe the pre-clinical evaluation of vaccines following to recognize the adverse effects. Following pre-clinical assessment outcomes, attributes of the target population, and understanding of related vaccines or other vaccines including the same adjuvants help to observe the safety assessment during the clinical experiment. Due to the restricted number of subjects, infrequent uncommon adverse events may not be recognized before authorization. Due to this reason for the continuous testing and observation of vaccine safety in the large population, the post-approval surveillance procedure needs to be established [46].

Observing adverse events needed particular surveillance during clinical development or post-approval by using pre-clinical experiments in suitable animal species. The data available about the adjuvant's toxicity during the pre-clinical and clinical studies may provide some additional information by recognizing the mechanism of action of a particular adjuvant. A small number of studies have thoroughly examined a specific adjuvant toxicity mechanism; however, great effort has been made to explore the mechanisms of action of adjuvants, importantly those mechanisms being responsible for the immunostimulating effects as well as for adverse effects. Because of the different varieties of the adjuvants, it is very difficult to analyze a reductionist inspection of potency/toxicity. Therefore, each analysis must be achieved following a specific adjuvant. One of the major challenges for the formulation of future adjuvants and adjuvanted vaccines is the recognition of suitable biomarkers and bio-models with the capacity to analyze potency, immunogenicity, toxicity, and subject-specific signatures (e.g., genetic makeup) [47]. Such information may be used to continuously determine the benefit-risk outline of the vaccine adjuvant during its biological clock and can also regulate the formulation of new vaccines. Rapidly emerging approaches to the rational design of adjuvants are committed to improving the potency, safety and immunogenicity of future vaccines.

The Future for Vaccine Adjuvants

Globally each year billions of vaccine doses are administered not only to mature individuals but also to small children so, vaccines are recognized as one of the most potent and effective sources against emerging infectious diseases for ensuring human health. Conventional methods of vaccination utilized inactivated or whole attenuated pathogens which were highly effective yet reported many side effects. This is one of the major explanations that current vaccines are highly safety-profile based on a rational design and extremely purified recombinant protein/peptide antigens, but with less immunogenicity. The principal aim of novel vaccine development is the introduction of adjuvants to enhance immunogenicity. However, such a vaccine composition evokes a question about the safety issues of vaccine adjuvants. The activation of undesirable immune feedback due to the potential of vaccine adjuvants should not be abandoned and research should be performed to make sure of the safety issues of adjuvants.

Currently, different new adjuvants are being evaluated in both pre-clinical and clinical experiments [48]. With the use of current adjuvants, many of the new approaches have a unique target in the innate immune network to stimulate adaptive immunity as well as the efficacy of vaccines. Recently by enhancing immunogenicity and antigen delivery, different studies expressed the antimicrobial peptides and cell-penetrating peptides to adjuvant vaccines [49]. To enhance the immune feedback to COVID-19 vaccines probiotics are also being evaluated as possible oral supplements [50]. In the adjuvant design, the discovery of TLRs has introduced a revolution. While in current clinical vaccines, there are TLR4 and TLR9 agonists, other TLR agonists are applicable too as they can influence different immunological mechanisms.

TLR5 is a cell surface PRR with one of the most optimizing emerging targets that identify a broadly conserved amino acid sequence on the flagellin of bacteria [51]. Mobilization of chemokine production and dendritic cells leads to the stimulation of TLR5, as well as strong humoral feedback but minimal cellular feedback [52]. For vaccines targeting pathogens such potential for humoral immunity favors this adjuvant as being attractive. TLR7 and TLR8 are also optimizing emerging targets that are present in the endosome and perceive RNA and small molecules, such as imiquimod [53]. TLR7/8 triggers both cellular and humoral immunity when activated with imiquimod or similar small-molecule agonists. Unfortunately, such molecules also demonstrate high toxicity and low water solubility [54]. However, the immunogenicity, safety, and solubility of these adjuvants can increase with the delivery catalysts such as nanofibers, liposomes, or nanoparticles [55]. For improving the immune feedback, other different PRRs are progressive targets for co-delivered antigens such as stimulators of interferon genes (STING), and C-type lectin receptors (CLRs) [56].

Saponins including QS-21 have played a significant effective role in antigen-specific immune feedback. Recently as a major component of the combination, adjuvants have been authorized for use in human vaccines, e.g., AS01b in Shingrix® for herpes zoster [57]. Regardless of their efficacy in clinics and research, the molecular and cellular methods of QS-21 and other related saponin adjuvants are inadequately recognized. For the use of QS-21 in different combinations and different formulations with other adjuvants greater efforts are required to investigate its mechanisms. Moreover, increased research activities toward immunological mechanisms and their manipulation have substituted empirical with the rational design of adjuvants that enhance and regulate the immunogenicity toward the appropriate part of the pathogen ultimately enhancing the potential of the safe vaccine [58, 59]. Hopefully in the future, such increased potential will confirm the safe, effective, and strong immunogenicity of upcoming novel vaccines.

CONCLUSION

Modern vaccination technologies are produced in a precise and hygienic way to provide a safe and effective protection against pathogens, while there is a dire need to provide an adjuvanted vaccine that maintains the potency of such vaccines, yet tolerating targeted immune activation. Adjuvants boost the immunogenic feedback of antigens and increase the effectiveness of vaccines. Adjuvants involved several modes of action such as regulation of antigen presentation by MHC molecules, localization of immune cells, induction of inflammasomes, and immunomodulation. Advances in immunology have raised many questions regarding the adjuvant's mechanism of action and its safety issues. However, for the development of safe and potential vaccines, knowledge of the adjuvant's side effects and virulence is essential. To reduce the toxicity of adjuvants and develop some novel adjuvants for enhancing the immune mechanism of action better understanding of the knowledge is required. Furthermore, current and future adjuvants will play a significant role as immunotherapies specifically for cancer treatment.

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