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Research Article

Adjuvant Option for Effective SARS-CoV-2 Vaccine

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ABSTRACT

Coronavirus Disease 2019 (COVID-19), caused by SARS-CoV-2 infection, has spread all over the world as an emerging infectious disease, causing a very serious impact on the global economy and public health. To date, there is no specific treatment or vaccines against coronaviruses. To prevent the infection and stop the pandemic, the research and development of effective SARS-CoV-2 vaccines has become an urgent matter. It is worth mentioning that appropriate adjuvants could enhance the strength and speed of the immune responses to vaccination. However, it takes time to customize different adjuvants with distinct targets (cellular or humoral immunity) and action intensity according to different antigens. In this review, we summarize the mechanism, advantages and disadvantages of adjuvants commonly used in licensed vaccines, as well as some novel adjuvants under development, hoping to provide information and increase the chances of success in developing effective SARS-CoV-2 vaccines in a short time frame.

Keywords: SARS-CoV-2; COVID-19; adjuvant; vaccine

INTRODUCTION

In December 2019, the outbreak of unexplained pneumonia patients appeared in Wuhan, China. Soon the pathogen causing this disease was identified to be the genus Betacoronavirus (β-CoV) of the family Coronaviudae. This coronavirus disease and the pathogen were officially named as COVID-19 and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), respectively. So far, the ongoing epidemic is spreading all over the world, and the number of confirmed cases in the world is increasing by tens of thousands every day, which is difficult to control in the short term. As of 19 June, the virus has caused over 8 million confirmed cases and over 450,000 confirmed deaths worldwide [1]. The global pandemic of COVID-19 has caused serious public security problems and we are still in the middle of this pandemic. Although some countries have taken control of the infection due to the implementation of restrictive measures, scientists are working as hard and fast as possible to develop prevention methods and therapeutics to curb the pandemic and prevent the next outbreak. Intense efforts have been directed to develop vaccines, the most effective and economical means to prevent and control infectious diseases [2]. Given the urgency of an effective vaccine more than 140 vaccines have been at different stages of development, including DNA-, RNA- based formulations, recombinant-subunits containing viral epitopes, adenovirus- based vectors and purified inactivated virus.

Adjuvant is a kind of non-specific immune enhancer. It can not only enhance the immunogenicity of the vaccine, effectively reduce the amount of antigen used, but also significantly improve the immune response to the target antigen by delaying the release of antigen, increasing the recruitment of cells at the injection site, enhancing the uptake of antigen presenting cells (APCs) and other mechanisms [3]. This is of great significance to the research and development of the vaccines. With the continuous advancement of science and technology, the types of vaccine adjuvants are also constantly enriched. According to their chemical composition, they can be roughly divided into aluminum-containing adjuvants, protein adjuvants, nucleic acid adjuvants, lipid-containing adjuvants, mixed adjuvants and other categories. However, due to the limitations of safety and effectiveness, most adjuvants are still in clinical trials. To date, the vaccine adjuvants approved for human use mainly include aluminum-containing adjuvants, MF59, AS01, AS03, AS04, etc. Here, we hope to provide some information for the development of SARS-CoV-2 vaccines by summarizing the adjuvants that commonly used in licensed vaccines (Table 1).

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Table 1: Adjuvants discussed in this review.

Adjuvants	Components	Type	Licensed vaccines
Aluminum adjuvants	aluminum hydroxide /aluminum phosphate	aluminum-containing adjuvants	diphtheria-pertussis-tetanus vaccine, human papillomavirus vaccine, hepatitis B virus vaccine, etc.
MF59	squalene, tween80, span85	oil-in-water emulsion	influenza vaccine
AS01	MPL, QS-21	liposome	malaria vaccine, herpes zoster vaccine
AS02	MPL, QS-21	oil-in-water emulsion	-
AS03	α-tocopherol, squalene, polysorbate 80	oil-in-water emulsion	influenza vaccine
AS04	MPL, aluminum hydroxide/aluminum phosphate	Aluminum adjuvants and immunostimulant	human papillomavirus vaccine, hepatitis B virus vaccine
Virosomes	phospholipid, viral spike glycoproteins, other viral proteins	liposome	influenza vaccine, hepatitis A virus vaccine
ISA51	mineral oil, surfactant	water-in-oil emulsion	non-small-cell lung cancer vaccine
CpG ODN	cytosine, guanine	DNA	hepatitis B virus vaccine

ALUMINUM-CONTAINING ADJUVANTS

Aluminum-containing adjuvants were the first to be discovered and approved for human use. It is widely used in the production and development of various vaccines, including diphtheria-pertussis-tetanus vaccine, human papillomavirus vaccine, hepatitis B virus vaccine and so on [4]. Aluminum-containing adjuvants mainly include aluminum hydroxide (Al(O)OH), aluminum phosphate (Al(OH)x(PO4)y) and aluminium potassium sulfate ($KAl(SO_4)_2 \cdot 12H_2O$), of which the first two are widely used, especially aluminum hydroxide adjuvant [5]. According to previous studies, aluminum adjuvants play a role through the depot effect, that is, the aluminum adjuvants can delay the release of the antigens in vivo after the antigens are absorbed through certain physical or chemical actions [6]. By doing so, it could provide continuous stimulation to the body, promote more adequate and effective contact between antigens and immune cells, and induce the body to produce robust immune responses. However, in the light of the recent studies, it has been found that this mechanism does not seem to be a favorable evidence for the adjuvanticity [7-9]. Studies have shown that the combination of aluminum adjuvants and antigens to form particles is conducive to phagocytosis by APCs, such as macrophages and dendritic cells (DCs), thereby enhancing the immune response to antigens [10]. Aluminum adjuvants can also induce inflammatory responses by targeting nucleotide binding oligomerization domain (NOD) like receptor protein 3 (NLRP3, also named as NALP3). It can recruit and activate APCs, thereby promoting antigen uptake, processing and presentation, regulating innate and acquired immune responses, and exerting adjuvant activity [10,11].

Aluminum adjuvants are the longest and most widely used adjuvant in humans. After decades of practice, it has been proved that they are safe, effective, low-cost and easy to operate. The combination of aluminum adjuvant and vaccine can significantly improve the innate immune response and humoral immune response, induce the activation of CD4+ T cells and stimulate B cells to produce related antibodies [8]. However, aluminum adjuvants also have some disadvantages. First, the immune response induced by aluminum adjuvant vaccine usually shows obvious Th2 bias and weak induction of cellular immunity [12]. Thus, this immune response is effective against extracellular pathogens, but ineffective against intracellular pathogens. Second, the specific immune response possibly promotes the secretion of IgE, which has the risk of causing allergic diseases [13]. Third, some studies have shown that vaccination with aluminum adjuvant vaccines can cause certain adverse reactions, such as erythema, subcutaneous nodules, granuloma, pain, and neurological diseases [10,14,15]. Fourth, the aluminum salt grid structure that adsorbs and supports the antigen is easily destroyed when frozen, so the aluminum salt adjuvant cannot be refrigerated [16,17]. Gao et al. developed inactivated virus-based vaccine (PiCoVacc) plus alum, which was the first to be reported efficacy against COVID-19 in non-human primates [18].

MF59

MF59 is the second adjuvant to be marketed after the aluminum adjuvants, and it is the first oil-in-water emulsion approved as a human adjuvant. It has been used in seasonal influenza vaccines and pandemic vaccines [19]. MF59, with squalene, tween 80, span 85, and citric acid buffer as the main components, is a tiny stable droplet with a diameter of about 160 nm, which has good safety and tolerance [20]. The main mechanism of MF59 is to activate the cells at the injection site, up-regulate the expression of cytokines and chemokines, so as to further recruit monocytes, granulocytes, and other immune cells, accelerate the differentiation of monocytes into DCs, and enhance the uptake of antigens and transport to the draining lymph nodes [21-23]. Related studies have shown that MF59 can also promote the retention of antigen in lymph nodes and follicular dendritic cells, which plays a vital role in the development of adaptive immune responses [24]. MF59 can rapidly induce innate immune response at the injection site and draining lymph nodes, promote antigen uptake, and then activate the B cell response mediated by CD4+ T follicular helper (Tfh) cells to induce the production of specific immune response to the pathogens [22,25,26]. It is worth mentioning, Naru Zhang et al. identified different adjuvants for subunit vaccines based on receptor-binding domain of Middle East respiratory syndrome coronavirus (MERS-CoV) and found that MF59 is the most potent as judged by its superior ability to induce the highest titers of IgG, IgG1 and IgG2a subtypes, and neutralizing antibodies. The addition of MF59 significantly augmented the immunogenicity of subunit vaccine candidate of MERS-CoV to induce strong IgG and neutralizing antibody responses as well as protection against MERS-CoV infection in mice, suggesting that MF59 is an optimal adjuvant for MERS-CoV RBD-based subunit vaccines [27]. It seems practicable for the research and development of subunit vaccine of SARS-CoV-2.

Other groups also have shown that the MF59 adjuvant vaccines have good tolerability and high immunogenicity [28, 29]. More importantly, MF59 can induce the Th1, Th2 type immune response and the stronger antibody response than aluminum adjuvants [19,30,31]. In a study of staphylococcus aureus vaccines, the MF59 adjuvant staphylococcus aureus vaccine was found to induce sustained humoral and cellular immune responses [32]. Studies have also shown that MF59 adjuvant can cause adverse reactions including redness and swelling at the injection site, pain, fever, irritability, and loss of appetite, but they generally last for a short time and cause mild symptoms [33-36].

AS ADJUVANTS

Adjuvant System (AS) is a new series of adjuvants developed by GlaxoSmithKline Biologicals (GSK), mainly including AS01, AS02, AS03, and AS04. AS01 is a liposome-based adjuvant containing 3-O-desacyl-4'-monophosphoryl lipid A (MPL) and QS-21, it has been used for malaria vaccines and herpes zoster vaccines [37, 38].

MPL and QS-21 are immunostimulants, and both can be used alone as immunostimulatory adjuvants. MPL can activate APCs by binding to toll-like receptor 4 (TLR4), stimulate the secretion of cytokines and the expression of co-stimulatory molecules, enhance adaptive immune response, and promote antibody isotype switching [39-41]. In addition to stimulating the production of specific antibodies, QS-21 can also induce specific cellular immune responses and participate in the elimination of intracellular pathogens [38,42]. AS01 can recruit neutrophils and monocytes, promote the differentiation of monocytes into DCs, and enhance the antigen presenting ability of the injection site and draining lymph node DCs, but cannot increase the uptake ability [39,43], so as to activate CD4+ T cells and enhance the antigen-specific cellular immune response and humoral immune response.

However, the CD8+ T cell response induced by AS01 adjuvant was not found in preclinical models [37]. Other groups have shown that the synergistic effect of MPL and QS-21 can make AS01 induce stronger adaptive immune response [44]. AS02 is an oil-in-water emulsion containing MPL and QS-21, and it is the only adjuvant that has not been approved among the 4 AS adjuvants. AS03 is an oil-in-water emulsion containing α-tocopherol squalene, and polysorbate 80, which was used in influenza vaccines. But this vaccine has been recommended to be discontinued, because of the high risk of narcolepsy [45-50]. AS03 can induce the body to produce high-titer specific antibodies against the target antigen by activating the innate immune response [43,51]. AS03 and MF59 are oil-in-water emulsions, and both are used in influenza vaccines. Clinical trials have shown that AS03 has a better protective effect than MF59 [52], but it has not shown a significant advantage in pediatric trials [53]. AS04 is an aluminum adjuvant containing MPL, which has been used for human papillomavirus vaccines and hepatitis B virus vaccines [39,54]. AS04 was prepared by adsorption of MPL onto aluminum hydroxide or aluminum phosphate. Compared with aluminum adjuvant, AS04 adjuvant can induce a higher level of humoral immune response [55].

AS adjuvants also have a certain degree of adverse reactions, such as injection site pain, redness, swelling, fever, fatigue, myalgia, and headache [34,35,56].

VIROSOMES

Virosomes are liposomes made of phospholipid and viral spike glycoproteins or other viral proteins, which can be prepared from the envelope proteins of various viruses, such as influenza virus, respiratory syncytial virus (RSV) [57-59]. As a platform technology, influenza virosomes have been used as carriers and adjuvants for subunit vaccines [60]. They are recombinant virosomes made in vitro by simulating the natural structure of the virus, with a diameter is about 150 nm, free of viral nucleic acid [61], and are mainly composed of natural phospholipid, phosphatidylcholine, hemagglutinin (HA) and neuraminidase (NA) [62]. As promising novel vaccine adjuvants in the development of vaccines, influenza virosomes have been used in influenza vaccines (Inflexal® V) and hepatitis A virus vaccines (Epaxal®).

Virosome adjuvants can be used as a delivery system to deliver large molecules such as antigens, nucleic acids, and drugs [63]. By encapsulating antigens in virosomes, they can reduce the degradation of antigens outside the cells and deliver them to the lymph nodes through binding to cell receptors and membrane fusion to introduce antigens into the cytoplasm. Virosome adjuvants can also assist APCs to acquire antigens and present them in the form of major histocompability complex (MHC) I and MHC II [61], thereby activating CD4+ T cell and CD8+ T cell, enhancing the specific humoral and cellular immune responses [64,65]. This is very important for the development of vaccines for intracellular infectious pathogens such as SARS-CoV-2. Virosome adjuvant vaccines have good safety, tolerability and immunogenicity, but some adverse events are still inevitable [66,67]. Currently, virosomes have already been used as carriers and adjuvants for the vaccine development and cancer prevention and immunotherapy. For example, cervical cancer is one of the cancers successfully treated by virosome-fomulated cervical cancer vaccines [68].

MONTANIDE ISA51

Montanide ISA51 is a water-in-oil emulsion developed by Seppic, France. It consists of a mineral oil and a surfactant [69]. The non-small-cell lung cancer vaccine, developed with the ISA51 adjuvant, has been approved for clinical treatment of cancer patients [70]. After immunization, this vaccine can promote the production of specific anti-epidermal growth factor (EGF) antibodies, thereby reducing the concentration of EGF in the blood, blocking its binding to the EGF receptor on the tumor surface, and then inhibiting tumor growth [71,72]. Studies have shown that ISA51 adjuvant can improve the titers of specific antibodies and the responses of cytotoxic T lymphocytes (CTL) when mixed with antigens [73], suggesting that ISA51 could be considered as a vaccine adjuvant for intracellular pathogens. Other researchers have shown that the ISA51 adjuvant vaccines also have some adverse reactions, such as injection site pain, fever, fatigue and gastrointestinal diseases [69].

CpG ODN

Unmethylated cytosine - phosphorothioate - guanine oligode oxynucleotides (CpG ODNs) is the very promising adjuvant discovered in recent years. The hepatitis B virus vaccine prepared with CpG1018 adjuvant was approved by the US in 2017 [74], which is the first CpG ODN adjuvant vaccine approved in the world.

According to the structure and biological characteristics of CpG ODN, it can be divided into four types: type A (or D), type B (or K), type C and type P [75,76]. CpG1018 is type B CpG ODN. Some research group shown that CpG ODN is the agonist of toll-like receptor 9 (TLR9), which is mainly expressed in plasmacytoid dendritic cells (pDCs) and B cells [75,77]. By binding to TLR9, CpG ODN can lead to the immediate recruitment of MyD88, thereby activating downstream signaling molecules. These activation pathways can activate nuclear factor-kB (NF-kB) and activating protein 1 (AP1), thereby stimulating the Th1 type immune response and inducing B cell proliferation and antibody production [78, 79]. CpG ODN can also improve the function of APCs and promote the production of antigen-specific humoral and cellular immune responses [75]. Additionally, other group has showed that CpG ODN can improve the immune function of immunocompromised people [80,81]. Although CpG-ODN has good safety and tolerability, it can still cause mild to moderate adverse reactions, including local reactions at the injection site, flu-like symptoms, etc [82]. Briefly, the main mechanisms and by-effects of the above adjuvants are summarized in Table 2.

OTHER ADJUVANTS

With the in-depth study of the immune system, some key signaling molecules in the immune signaling pathways have become common targets in the development of adjuvants, such as stimulator of interferon genes (STING), Toll-like receptors (TLR), C-type lectin receptors (CLR), NOD-like receptors (NLR), etc. These novel adjuvants that are still in the development have been proved to have remarkable effects in enhancing the immune response.

Recently, the research teams of China and the United States have discovered a novel adjuvant, the pulmonary surfactant-biomimetic nanoparticles (PS-GAMP), which encapsulates 2′, 3′-cyclic guanosine monophosphate-adenosine monophosphate (cGAMP) in liposomes. PS-GAMP can activate the STING pathway in alveolar macrophages (AMs) and pulmonary epithelial cells (AECs), inducing the production of Type I interferons (IFN-Is) by intranasal immunization. It can also promote the vaccine to induce a broad-spectrum immune response against heterosubtypic influenza viruses [83]. Adequate contact of PS-GAMP with the lungs is essential to stimulate a stronger immune effect from respiratory virus vaccines. Moreover, the cross-protection of this adjuvant has a positive reference value for the development of beta-coronavirus lineage B broad-spectrum vaccines.

GLA-SE consists of a stable oil-in-water emulsion (SE) and glucopyranosyl lipid adjuvant (GLA) that is a TLR4 agonist [84]. It is well known that the decline of immune function in the elderly is the main contributor for the poor efficacy of vaccines in this population [85]. The strength of vaccine efficacy in the elderly is related to the ability of the vaccine to stimulate the CTL response required for disease prevention. GLA-SE, used in influenza vaccines, has been shown to enhance Th1 cell-mediated CTL response and has the potential to enhance protection against influenza virus in older adults [86]. This is important for the development of SARS-CoV-2 vaccines, because of the high mortality rate of COVID-19 in the elderly. TFPR1 is the PR-1 domain of triflin, which is a novel protein and peptide adjuvant recently discovered. It can act as an adjuvant by activating TLR2. Furthermore, animal experiments show that TFPR1 can enhance Th1-biased antibody- and cell-mediated immune responses in mice immunized with protein antigens or peptide antigens [87]. Currently, licensed human adjuvants are generally not effective in enhancing peptide-induced immune responses. Therefore, TFPR1 is promising to provide a choice for vaccine design, especially peptide-based vaccine. Besides, Trehalose-6, 6-dibehenate (TDB) is a CLR ligand, which can induce strong Th1 and Th17 immune responses. DDA/TDB (also known as CAF01) can induce mucosal and systemic antibody responses and has clinical antiviral effects [88,89]. In addition, the use of NLR ligands as adjuvants is also under research, and it is found that encapsulating NOD receptor agonists in Poly (Lactic Acid) nanoparticles (PLA-NPs) can highly activate the NF-κB pathway. The NOD ligands can be effectively taken up by DCs and promote DCs to secrete proinflammatory cytokines. Furthermore, co-injection of NOD ligand encapsulated by PLA-NPs with p24 antigen can significantly improve antibody response [90,91]. These novel adjuvants are not licensed, so their risks should be fully considered when designing vaccines.

CONCLUSION

The COVID-19 epidemic is still spreading around the world, and it may become a seasonal disease coexisting with humans for a long time. The development of vaccines is the most favorable means to completely stop the spread of SARS-CoV-2. The application of adjuvant is of great significance in the development of vaccines. It can not only effectively reduce the amount of antigen used, but also regulate the intensity and change the type of immune response. In this paper, we reviewed the commonly used vaccine adjuvants, including aluminum adjuvants, MF59, AS adjuvants, virosomes, ISA51, CpG ODN and other promising adjuvants, hoping to assist researchers to choose appropriate adjuvants according to the R & D needs of different vaccines and provide information for the development of SARS-CoV-2 vaccines, so as to develop more safe, economical and effective SARS-CoV-2 vaccines for the benefit of human health.

Table 2: The main mechanism and adverse reactions of licensed adjuvants.

Adjuvants	Immune mechanism	Adverse reactions
Aluminum adjuvants	pro-phagocytic effect, pro-inflammatory NLRP3 pathway	erythema, subcutaneous nodules, granuloma, pain, neurological diseases, IgE-mediated hypersensitivity
MF59	activate and recruit immune cells, enhance the differentiation of monocytes into DCs	redness and swelling at the injection site, pain, fever, irritability, loss of appetite
AS01	TLR4 agonist	injection site reactions, myalgia, fever
AS03	activate and recruit immune cells	injection site pain, narcolepsy, fever, headache, arthralgia
AS04	TLR4 agonist	injection site pain, fatigue, headache
Virosomes	antigen presentation	injection site pain
ISA51	activate and recruit immune cells	injection site pain, fever, fatigue, gastrointestinal diseases
CpG ODN	TLR9 agonist	local reactions at the injection site, flu-like symptoms

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