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An Overview of Adjuvants and Their Interaction with the Immune System

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Abstract

Vaccines have been under development for over two centuries and have significantly contributed to the decline in infectious diseases and mortality rates by eliciting targeted immune responses against pathogens. Adjuvants, while typically non-immunogenic, play a vital role in modulating immune responses when combined with vaccines, reducing the necessary vaccine dosage and enhancing immune memory. Generally, vaccines are formulated with appropriate adjuvants to strengthen the immune response to the vaccine antigen and to assess their potential in preventing disease spread. Additionally, adjuvants are crucial in steering both humoral and cell-mediated immune responses to foster pathogen-specific immunity. There is an increasing emphasis on utilizing advanced technologies to develop novel vaccines aimed at problematic pathogens, particularly those that show limited efficacy with conventional vaccines and outdated production techniques. An ideal adjuvant should exhibit minimal to no adverse effects and ensure safety for both short-term and long-term applications. This article provides a concise overview of adjuvants, examining their significance in autoimmune diseases, especially concerning disease progression and related challenges. Acknowledging the growing skepticism surrounding vaccines in recent years is important, with some research supporting this perspective. Furthermore, we explore the function of adjuvants in cancer vaccines, categorized as therapeutic rather than preventive, noting the substantial advancements achieved in this area.

Keywords: Adjuvants, Vaccines, Infectious disease, Cancer vaccine, Therapeutic vaccine

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Introduction

Vaccines have been being under development for over two centuries and have significantly contributed to the decline of illness and death caused by infectious diseases (1). The initial adjuvanted vaccines were produced during the 1920s; however, from then until the 1990s, there was minimal advancement in developing novel adjuvant vaccines. This lack of progress can be attributed, in part, to a limited comprehension of innate immune signaling, which varies across different species. In recent times, several novel adjuvants have been discovered for use in human vaccines. These include AS01B in the latest shingles vaccine, 5'—C—phosphate—G—3' (CpG) in the new Hepatitis B vaccine, and MF59 in the adjuvant flu vaccine (2).

Indeed, the utilization of adjuvants has the potential to decrease the quantity of purified antigens necessary for adequate immunization,

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thereby enhancing the economic viability of vaccine production. It may be feasible to combine newly developed synthetic immunoregulators, which have a relatively low molecular weight and are non-toxic, with antigens to regulate specific immune system compartments. However, the issue of adjuvant safety remains unresolved and is a significant barrier to the systematic advancement of adjuvanted vaccines. The concern of inducing cancer or other immediate and long-term disruptions to the immune system must be addressed through meticulous and logical experimentation and the establishment of appropriate guidelines for human studies (3).

In vaccinating healthy individuals, it is crucial to ensure that vaccines cause only a few mild side effects or, ideally, no side effects. However, adjuvant active substances can still serve as valuable tools for studying the immune system, even though they may accompanied by vaccination side effects by excessively stimulating the immune system. The understanding of adjuvant side effects is derived from investigating highly reactive adjuvants and observing significant overdosing of traditional adjuvants. Local reactions that may occur after using such adjuvants can range from localized pain and redness to the formation of granulomas, cysts, abscesses, and ulcers. These reactions are more likely to occur if the adjuvant is overdosed beyond acceptable limits. Adverse systemic reactions, such as pyrogenicity, flu-like symptoms, and autoimmune disorders, can also result from adjuvant- or cytokine-induced immune system stimulation. However, these reactions disqualify the practical use of the adjuvant in vaccination (4).

Experimental studies have unequivocally demonstrated that adjuvants such as Alum can trigger significant immunological disorders in human beings. Specifically, the presence of aluminum in adjuvant form poses a potential threat to the development of autoimmunity, chronic inflammation in the brain, and related neurological complications. Consequently, such adjuvants may have extensive and detrimental health implications (5). However, using adjuvants like alum in conjunction with the desired pathogen alone does not elicit adequate immune responses. It often skews the responses towards T-helper 2 (Th2) cell polarization. Therefore, it becomes imperative to incorporate alternative substances alongside alum to generate an appropriate immune response. This requirement appears to be essential (6, 7).

It is worth noting that the latest licensed adjuvants appear to be Alhydroxiquim-II and CpG ODN (1018 ISS), which were utilized in coronavirus disease 2019 (COVID-19) vaccines COVAXIN and CorbeVax in 2022. The former is in the form of Alum adsorbed to a toll-like receptor (TLR) 7 and TLR8 agonist, facilitating the transport of small molecules of Alhydroxiquim-II to lymph nodes, where they detach from alum to activate the two cellular receptors TLR7 and TLR8. The latter is a soluble TLR9 ligand (oligonucleotide) that is adsorbed to alum, resulting in increased cellular and humoral immunity and significant expression of T helper (Th) 1-specific cytokines. Notably, numerous adjuvants are currently in Phase 1 and 2 clinical trials, with their results and approval status expected to be available soon. Recent clinical studies involving novel adjuvants suggest that a combination of new immune potentiators or stimulators should be incorporated into future vaccine formulations for human application. The availability of these advanced adjuvants in various combinations will facilitate the development of effective vaccine strategies. While there is significant potential to enhance vaccine efficacy by incorporating innovative adjuvants that can provide improved immunity, these vaccines may also prove effective against pathogens exhibiting considerable antigenic diversity (7).

This article has gained increasing significance in various communities, particularly in the

wake of the extensive vaccination campaign for COVID-19. It raises critical questions regarding the potential link between vaccines and the onset of autoimmune diseases, as well as their role in preventing such conditions. Furthermore, there is an ongoing discussion about the safety of vaccinations for children. The article explores the possibility that possible adverse reactions associated with vaccines may be attributable to adjuvants. It specifically examines the influence of adjuvants on the development of autoimmune diseases and the implications for pediatric vaccination. The Bacillus Calmette-Guerin (BCG) adjuvant is highlighted as a notable exception compared to others, and the article also addresses the function of adjuvants in cancer vaccines, which are categorized as preventive vaccines. Additionally, the discussion encompasses the topic of COVID-19 vaccines, which continues to be a global concern. The article also considers the design of innovative vaccines utilizing new adjuvants, which presents a promising outlook despite concerns regarding immunogenicity and potential long-term toxicity.

Adjuvants and Immune System

The role of Adjuvants in Autoimmune Diseases

Vaccines are medications administered to healthy individuals. Like other medicines, vaccines can result in side effects. These side effects are typically temporary and short-lived. However, in rare cases, they may cause hypersensitivity and lead to autoimmune reactions that can be severe and even fatal (8) (Figure 1). Scientific research has revealed that vaccine adjuvants, particularly aluminum compounds such as aluminum hydroxide and phosphate, can trigger autoimmunity (9).

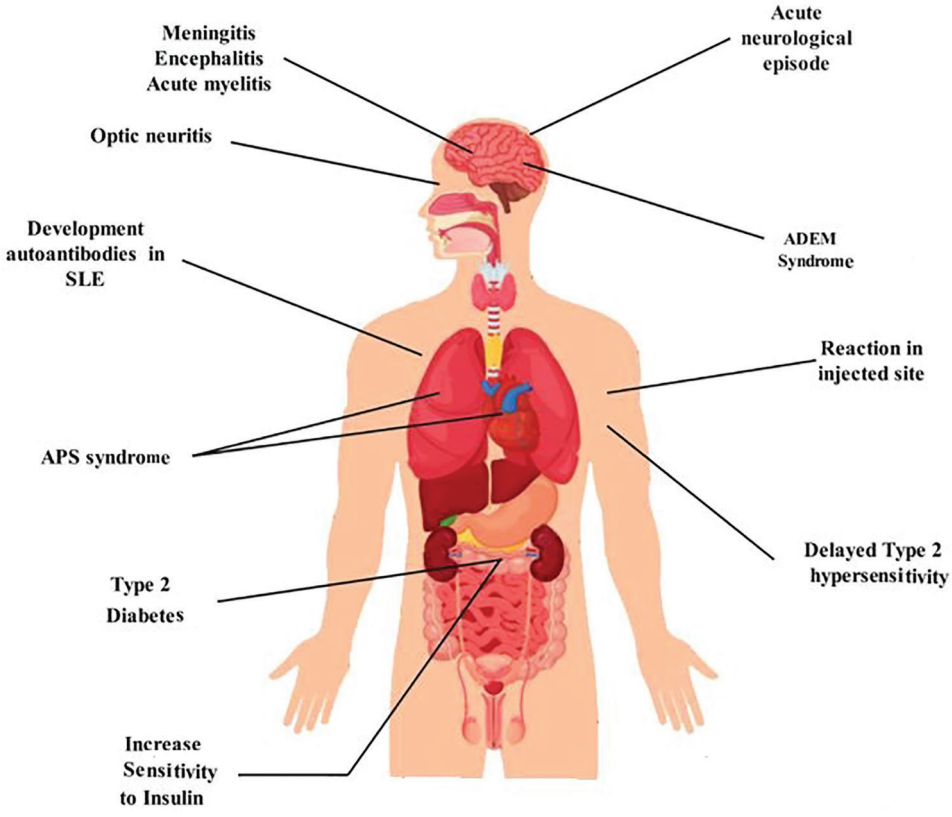


Figure 1. The side effects of vaccines, which manifest as autoimmune diseases in various parts of the body, appear to be associated with adjuvants. SLE: systemic lupus erythematosus, ADEM: Acute disseminated encephalomyelitis, APS: antiphospholipid syndrome XX

A newly identified syndrome called "Adjuvant-induced autoimmune/inflammatory syndrome" (ASIA) can cause various reactions, ranging from mild to severe. These reactions have been linked to irritation caused by excipients, including prolonged exposure to silicone, tetramethyl pentadecane, pristane, aluminum, infectious compounds, and other substances (10). Exogenous adjuvants can activate self-reactive T cells, thereby causing self-aggression. An example of this is an acute viral infection that precedes myocarditis in half of all cases. Human infectious myocarditis can be replicated in laboratory rat models (8). Lujan et al. have presented an intriguing model involving commercial sheep. After receiving repeated injections of an aluminum-containing adjuvant, the sheep exhibited an acute neurological episode with poor response to external stimuli and acute meningitis days after vaccination. The animals experienced a period of euphoria followed by weakness, extreme wasting, quadriplegia, and death. These symptoms have been suggested to be part of the ASIA syndrome spectrum. Moreover, biopsies of nerve tissue from laboratory animals have revealed the presence of alum (11).

The activation of the NLRP (Nucleotide-binding oligomerization domain, Leucine-rich Repeat, and Pyrin domain containing) 3 inflammasome pathway is one of the critical effects of aluminum excipients (12). This activation leads to the development of type 2 diabetes. Research using NLPR3 knockout mice has demonstrated that the lack of inflammasomes enhances glucose homeostasis and insulin sensitivity (13). Conversely, metals such as mercury, aluminum, nickel, and gold can induce immunotoxic effects in humans. The immunomodulatory effects of these metals include immunomodulation, allergic reactions, and autoimmune effects. They can act as immunosuppressants or immune adjuvants. Metals bind tightly to cells and proteins and can modify autologous epitopes (haptenization) (14).

Metal hypersensitivity may cause delayed type IV hypersensitivity reactions. Vaccination can trigger autoimmune responses and the development of autoantibodies (15). A disparity in autoantibody production has been observed between influenza vaccines with and without excipients in healthy individuals (8). It has been suggested that molecular mimicry can cause an autoimmune response following influenza vaccination. However, there is currently insufficient evidence to support the claim that influenza vaccines lead to the development of autoimmune diseases. In fact, studies have demonstrated that influenza vaccines are safe for patients with systemic lupus erythematosus (SLE) or rheumatoid arthritis (RA) and can even lower their risk of respiratory infections (16).

Research conducted by various groups has revealed an increase in the incidence of narcolepsy diagnosis following the administration of the influenza vaccine, especially the PandemrixTM vaccine, which contains the ASO3 adjuvant. This correlation has been observed primarily in Finland, particularly among children aged 4-19 years, and through case reports from other nations (17); however, other studies have found no connection. Influenza A virus subtype hemagglutinin1 (H1) and neuraminidase1 (N1) (H1N1) infection has been linked to disease development in China, but no such link has been found in Europe (8). It is important to note that the combinations discussed above are linked specifically to the PandemrixTM vaccine containing adjuvant ASO3. There is no evidence to suggest that other H1N1 vaccines, with or without adjuvant, are associated with the same outcomes. These findings indicate a connection between the PandemrixTM H1N1 vaccine and the development of narcolepsy.

Additionally, studies conducted on mice undergoing post-trial therapy for Alzheimer's disease (AD) using vaccines containing aggregates of fragments of the amyloid precursor protein Ab42 have demonstrated the development

of Acute disseminated encephalomyelitis (ADEM) (8). Research has suggested that the Hepatitis B Vaccine (HBV) vaccine's pathogenicity and potential for autoimmunity may be linked to cross-reactivity between antigens of HBV antigen (HBsAg), a fungal antigen, yeast, and other components found in the vaccine (18). The Human papillomavirus (HPV) vaccine has been shown to cause the development of autoantibodies in adolescent girls with SLE (19).

It is well-established that autoantibodies can develop many years before the manifestation of a complete autoimmune disease. Furthermore, genetics also determines the production of a specific autoantibody. The relationship between genetics, autoantibodies, and vaccines remains an intriguing area of research that could be further explored (15).

While adjuvant vaccines may cause more reactions at the injection site, they do not increase the risk of overall adverse events (20). The occurrence of vaccine side effects depends on the presence of excipients. Among intramuscularly vaccinated individuals without excipients, local reactions occur in 9% of cases, whereas subcutaneously vaccinated individuals experience responses in 24% of cases. In conjugate vaccines, local reaction rates increase to 50% (15). The *Clostridium tetani* (*C. tetani*) vaccine contains an inactivated tetanus toxoid and an adjuvant, usually aluminum hydroxide. The tetanus vaccine has been extensively studied, and the most common disease associated with it is antiphospholipid syndrome (APS). However, there have also been reports of central nervous system (CNS) complications, including optic neuritis, acute myelitis, and encephalitis (21).

The etiology of "undifferentiated connective tissue disease" (UCTD) remains unknown. Researchers have posited that UCTD may fall within the ASIA spectrum due to its similarity in symptoms and adjuvant-related pathogenesis (22). Notably, the multiple sclerosis (MS) rat experimental model, EAE, can only be induced by injecting Ab42 with a complete Freund adjuvant (23). This observation underscores the crucial role of adjuvants in inducing ADEM and autoimmune conditions. ASIA syndrome, identified in 2011 by Shoenfeld and Agmon-Levin, is characterized by a hyperactive immune response to adjuvants (17). The application of adjuvants in triggering autoimmune conditions extends to RA. For instance, injecting complete Freund's adjuvant (CFA) into a footpad of Wistar rats results in swelling in all footpads, even those not injected, within a few days. This phenomenon demonstrates a complex immunological response and the onset of autoimmune arthritis (24). Recent studies indicate that herbal and medicinal substances, including atorvastatin, nicotinic acid, and the conditioned medium of mesenchymal stem cells treated with caffeine, pentoxifylline, or prednisolone, have shown potential in alleviating the complications associated with autoimmune diseases triggered by adjuvant injection (24-26).

Adjuvant in Cancer Vaccines

Unlike conventional vaccines designed to prevent diseases, cancer vaccines aim to cure active disease. The only exception is the recently approved vaccine that prevents cancer caused by bacteria (27). Despite promising outcomes in preclinical research, there are significant challenges in developing cancer vaccines that have yet to be overcome in clinical settings. Currently, the Food and Drug Administration (FDA) has only approved one vaccine, sipuleucel-T, which is used to treat metastatic castration-resistant prostate cancer in a specific group of patients who experience minimal symptoms. The initial step in triggering an immune response involves antigen-presenting cells (APCs) sampling and presenting the antigen to the innate immune system. Therefore, effective cancer vaccine formulations include cancer-specific antigens combined with immunostimulating adjuvants that activate the APCs to overcome tolerance and

suppress adverse reactions. Adjuvant-stimulated dendritic cells (DCs) have emerged as promising candidates for efficient cross-presentation of tumor antigens. Alum has been shown to stimulate cross-presentation of tumor-associated antigen (TAA) in FDA-approved vaccine adjuvants (28) and several currently under-development adjuvants, including TLR3 I ligand: C and granular saponin excipient, ISCOMATRIX®. While adjuvants may promote the propagation of antigen-based determinants in the context of tumor antigens, they also pose potential risks. For example, administering a monoclonal antibody that blocks cytotoxic T lymphocyte antigen 4 (CTLA-4), commonly used in adjuvant cancer therapy, has been shown to promote the spread of antigenic determinants but may accelerate autoimmune disease. ISCOMATRIXTM, an adjuvant multicomponent "immune-stimulating complex" composed of cage-like structures of phospholipids, saponins, and cholesterol, has demonstrated efficacy in promoting epitope replication and maturation of antibody affinity when included in influenza vaccines and has been utilized as an adjuvant component in cancer vaccines. Furthermore, ISCOMATRIXTM has been shown to promote cross-presentation of tumor antigens. Similar adjuvant strategies may hold promise for increasing the efficacy of vaccines based on single epitopes. However, research on the role of adjuvants in this phenomenon and the importance of spreading determinants in tumor regression is still in its nascent stages. The selection of adjuvants for vaccine development strategies not only directly enhances anti-tumor immunity but also blocks checkpoints or inhibitory networks that could potentially eliminate tumors. TLR agonists such as 3-O-desacyl-4'-monophosphoryl lipid A (MPL), while serving as potent adjuvants in some cases, also have the effect of elevating Tregs (29).

In the absence of adjuvant antigens targeted to immature DCs, inflammation or microbial stimulation induces tolerance rather than a potent immune response. Effective adjuvants need to attract immune cells to the injection site while also promoting cell-mediated trafficking of antigens to draining lymph nodes and triggering the activation of APCs (27). Currently, water-in-oil emulsions, such as Montanide ISA 720 and Montanide ISA-51, have been widely adopted as adjuvants, forming a depot at the injection site. In a clinical trial, Montanide ISA-51 stimulated the production of CD4+ and CD8+ T cell responses in patients vaccinated with long oncoproteins E6 and E7 peptides.

To generate a more robust and longer-lasting immune response, new vaccine adjuvants targeting specific immune system components have been developed. Newer adjuvants consisting of pathogen-associated molecular pattern molecules (PAMPs) are now being used, as they provide a danger signal recognized by pattern recognition receptors (PRRs), thereby inducing an immune response. TLR agonists are increasingly being employed as vaccine adjuvants; they mimic microbial stimulation and have been shown to increase vaccine efficacy, particularly for cancer treatments (27).

Several TLR agonists are currently undergoing trials as adjuvants for cancer vaccines. One of the most commonly used is polyinosinic–polycytidylic acid with polylysine and carboxymethylcellulose (Poly-ICLC): a TLR3 agonist. Others include monophosphoryl lipid A (MPLA), a TLR4 agonist; imiquimod, a TLR7 agonist; resiquimod, a TLR7 and TLR8 agonist; and CpG oligodeoxynucleotide (CpG ODN): a TLR9 agonist.

Researchers are also investigating novel adjuvants to enhance the efficacy of cancer vaccines. These include CD40 agonists, which directly target antigens to the early endosomes of DCs and mediate cross-presentation. Although CD40 agonist antibodies have not been extensively studied as vaccine adjuvants in clinical trials, they have been studied

independently as monotherapy (27).

Another promising set of adjuvants is known as Stimulator of Interferon Genes protein (STING) agonists. STING, a transmembrane protein in the endoplasmic reticulum, can activate a type I interferon (IFN) response when triggered by intracellular DNA. These agonists include synthetic cyclic dinucleotide derivatives and cyclic di-guanosine monophosphate. Research has demonstrated their anti-tumor effects in mice. When used in a cancer vaccine, a STING agonist must be combined with an adjuvant or delivery system that targets only myeloid cells *in vivo* (27).

In addition to pathogen-derived molecules, researchers have discovered that specific cytokines can also serve as adjuvants. Immunostimulatory cytokines, including interleukin 2 (IL-2), IFN, IL-12, and granulocyte-macrophage colony-stimulating factor (GM-CSF), have been extensively studied for their potential as adjuvants. However, most current research has focused on their application in vaccines and cellular-based therapies. Among all the immunostimulatory factors, GM-CSF has been the most extensively studied and employed in numerous cancer vaccine trials. Unfortunately, clinical trials on GM-CSF have been largely unsuccessful, with only a few showing any clinical benefit. Preclinical studies have suggested that GM-CSF might expand myeloid-derived suppressor cells (MDSCs), which can suppress cell-mediated anti-tumor responses (27).

The anti-tumor response was generated by CD4+ T cells, and an increase in CD4+ tumor-infiltrating T lymphocytes (TILs) caused tumor regression (30). A recent study has shown that by combining various TLR-stimulating adjuvants with Modi-1 and GM-CSF (including TLR9/TLR4, TLR9, TLR3, TLR1/2, and TLR7 agonists), robust Th1 responses can be generated. The TLR1/2 AMPLIVANT® adjuvant elicited the strongest response of all the adjuvants. The efficacy of the AMPLIVANT® adjuvant is evidenced by its use in an ongoing study on two HPV-16 peptides in patients with head and neck squamous cell carcinoma. These findings highlight the importance of screening various adjuvants and doses to identify the optimal combination for inducing a powerful immune response. In 2021, the Modi-1 vaccine containing AMPLIVANT® adjuvant entered a Phase 1/2 clinical trial (27).

BCG as an adjuvant

In 1921, Albert Calmette and Camille Guerin presented a vaccine obtained by serial subculturing on glycerinated bile potato medium—the strain known today as BCG. BCG has shown great effectiveness in treating disseminated tuberculosis and tuberculosis meningitis. It is important to note that the BCG vaccine offers more than just protection against tuberculosis, owing to its various mechanisms of action, including immunomodulatory properties. However, it can be harmful to certain immunodeficient patients. There has been speculation that the BCG vaccine could be a potential diabetes treatment. Faustman's research demonstrated that the BCG vaccine could reduce blood glucose levels to a nearly normal range in individuals with type-I diabetes mellitus and improve their glycemic control. A study conducted on mice found that BCG can provide neuroprotection against Parkinson's disease and experimental autoimmune encephalomyelitis (31). Figure 2 illustrates the different effects of adjuvant BCG on components of the immune system and diseases such as autoimmune disorders and cancers.

Research has shown that BCG can have positive effects on cancer. Immunization with BCG may enhance the immune system and offer protection against cancer later in life. Animal studies have also demonstrated BCG's ability to inhibit tumor growth (32). Prior BCG immunization has been associated with increased survival in melanoma patients.



Figure 2. The different effects of adjuvant BCG on components of the immune system and diseases like autoimmune disease and cancers.

Despite its efficacy in non-obese diabetic mice, BCG vaccination is ineffective in preventing diabetes in humans (33, 34). Studies in a mouse model have suggested that BCG vaccination may prevent AD (35).

BCG immunotherapy is the most effective treatment for non-muscle-invasive bladder cancer, with over 3 million annual therapies administered. The mechanism behind intravesical BCG's antitumor activity is not yet fully understood, but it is believed to involve a local nonspecific immunological boost that attracts immunocompetent cells (36). When used to treat bladder tumors, BCG causes the tumor cells to release cytokines and chemokines, subsequently leading to their elimination by cytotoxic cells. Additionally, BCG can enhance the presence of major histocompatibility complex (MHC)-II and Intercellular Adhesion Molecule 1 (ICAM-1) molecules on the surface of tumor cells, rendering them more recognizable and vulnerable to attack. The National Comprehensive Cancer Network (NCCN) guidelines also recommend this treatment for some cases of inoperable stage III melanoma as an intralesional treatment. It is noteworthy that BCG vaccination has been shown to prevent hyperoxic lung injury in neonatal rats. Moreover, a recent meta-analysis suggests that early BCG vaccination may be associated with a reduced risk of leukemia. Furthermore, a retrospective review found that receiving BCG vaccination during childhood is linked to a lower likelihood of developing lung cancer.

An indirect diagnostic tool for Kawasaki disease (KD), an acute febrile illness affecting young children, can be provided by BCG. KD is a challenging diagnosis that relies on clinical signs and symptoms. Notably, erythema and induration of the BCG site are now recognized as significant clinical clues, first described by Kawasaki in 1970 (31). BCG site erythema has long been regarded as a specific sign of the disease, particularly in infants. In the late 1990s, studies found that children who received the BCG vaccine had a lower prevalence of allergic asthma. According to a longitudinal study, BCG vaccination can improve lung function and decrease the need for emergency asthma medication compared to a placebo. This theory, known as the hygiene hypothesis, suggests that vaccinating newborns with BCG can help relieve asthma symptoms in animals and humans by increasing the production of Th1 cytokines (37).

Adjuvants in infant vaccines

Protecting newborns from infections through vaccination poses a significant challenge, as they are less responsive than adults. Neonates struggle to produce high levels of antibodies, even when using adjuvants that typically stimulate a robust response in adults. However, there is a risk of vaccine-enhanced disease despite the use of adjuvants. Studies conducted on rodents indicate that an excessive Th2 response, exacerbated by the Th2 adjuvant alum, may be linked to vaccine-enhanced disease.

Effective neonatal vaccines may benefit from novel adjuvants, with particular emphasis on TLR agonists. While TLR2 and TLR5 ligands boost antibody production, TLR3, TLR7, TLR8, and TLR9 promote Th1 cell-polarized responses. The incorporation of adjuvants in neonatal vaccine formulas can potentially accelerate the development of the neonatal immune system (38).

Research has shown that adjuvants enhance the response of neonatal follicular helper T (Tfh) cells and augment the frequency and quality of germinal center (GC) and memory B-cell responses, resulting in increased production of high-affinity, neutralizing antibodies, and improved cellular immunity through Th1 cell polarization (39). However, there is a lack of data regarding whether adjuvants improve the immune response to neonatal vaccines in humans. While alum-adjuvanted HBV vaccines are effective for infants and adults, they prove ineffective for preterm infants. In a study involving 7-day-old mice, the squalene oil emulsion adjuvant MF59 failed to elicit protective influenza antibody responses, even after administering a second booster dose. Conversely, lipidated TLR7/8 agonists administered on the first day of life have been shown to improve B-cell responses to pneumococcal vaccines in rhesus monkeys (38).

Overall, the evidence suggests that adjuvants typically utilized for adults may not be as effective for neonates. Nonetheless, a few vaccinations, such as BCG, have proven successful for newborns. BCG comprises glycolipids, glycopeptides, and other sugar-based structures that effectively activate the innate immune system. Advax adjuvants, which are based on a plant polysaccharide called delta inulin, have shown encouraging outcomes in neonatal vaccines. It is worth noting that inulin must be transformed into delta inulin microparticles through crystallization to function as an effective adjuvant, as in its soluble form, inulin does not possess any discernible immunoactivity. When used alone or in combination with TLR agonists, Advax has demonstrated the ability to boost both humoral and cellular immunity. Additionally, the Advax adjuvant provided complete protection to 7-day-old mouse pups who were given a single dose of inactivated influenza vaccine, whereas the vaccine alone did not offer full protection (40).

The positive impact of sugar-based adjuvants, such as delta inulin, curdlan, and TDB, on neonatal vaccine responses in animal models underscores the potential for identifying enhancers of neonatal vaccine responses. To fully comprehend the unique advantages of these compounds, further research is required to investigate the variances in immune signaling pathways between neonates and adults (38).

Uncertainty still surrounds the safety of alum adjuvant for infants. According to the Centers for Disease Control and Prevention (CDC), a study was conducted to determine the amount of aluminum exposure from vaccines during infancy. The researchers discovered that the total amount was well below the minimum risk levels established by the Agency for Toxic Substances and Disease Registry (ATSDR). Despite this, there have been reports of chronic local granulomatous inflammation, known as macrophagic myofascitis, in a limited number of patients who received intramuscular vaccines containing aluminum. This condition is believed to be caused by an ongoing inflammatory response to the residual aluminum from the vaccination site, leading to neurological symptoms such as muscle pain, joint pain, chronic fatigue, weakness, and cognitive impairment. However, no evidence has been found linking aluminum adjuvants to chronic neurologic diseases (41, 42).

The potential link between aluminum content in vaccines and autism spectrum disorders in children has been a subject of debate. However, a large meta-analysis of cohort studies has shown no association with alum. A long-standing hypothesis suggests a connection between aluminum and the onset of AD. This theory posits that the accumulation of aluminum in the brain over time

could lead to symptoms akin to dialysis-associated encephalopathy. The most significant risk of aluminum exposure is associated with intravenous solutions used for delivering micronutrients during parenteral nutrition. Medical studies have indicated that preterm babies may suffer from lasting consequences due to aluminum exposure via injections. Consequently, it is crucial to limit the presence of aluminum, even though its complete elimination from current products may not be feasible. Additionally, patients with kidney ailments should avoid medications containing excessive amounts of aluminum, such as phosphate binders, to mitigate their exposure to this element (43).

Ensuring safety is paramount when developing vaccines for neonates and infants. There are legitimate concerns that potent adjuvant systems may increase the chances of reactogenicity, systemic inflammation, and autoimmune disorders. Exposing neonates and infants to proinflammatory stimuli, particularly those that trigger systemic inflammation, may pose a risk of perinatal brain injury due to repeated exposure to TLR2 agonists. To effectively activate cellular responses for diseases such as malaria, tuberculosis, and human immunodeficiency virus (HIV), it may be necessary to trigger multiple signaling pathways by combining TLR agonists or utilizing live vectors. The neonatal BCG vaccine contains numerous TLR agonists and has demonstrated encouraging safety and efficacy. However, due to a potential correlation between vaccination and autoimmunity, a comprehensive risk assessment is necessary, as causality has yet to be fully established (39).

The immune response in infants differs significantly from that of adults and undergoes substantial changes during the first few years of life. Unfortunately, current formulations have not yet been adjusted to address age-appropriate considerations. It is anticipated that a high ratio of IL-23 to IL-12 will be observed in infants by age one, which may increase the risk of a hyperinflammatory response favoring Th17. However, the production of IFN-I by myeloid cells dependent on interferon regulatory factor (IRF)3 and by plasmacytoid cells dependent on IRF7 tends to decrease early in life, potentially mitigating the associated risk. When developing safe and effective adjuvants, a primary concern is ensuring their action remains localized both spatially (at the site of topical administration) and temporally (39). Further research is needed regarding the use of adjuvants and vaccines in infants and newborns with special immune system conditions.

Adjuvants in COVID-19 vaccines

The establishment of a global repository of COVID-19 vaccines is crucial for widespread vaccination. Over the past two decades, novel adjuvants, such as Aluminum hydroxide, MF59, AS03, CpG 1018, and CoVaccine HT, have been incorporated into licensed vaccines. Consequently, the durability and effectiveness of vaccines depend heavily on appropriate adjuvants. Table 1 provides concise information

Table 1. Adjuvant used in COVID-19 vaccine

Adjuvants	Component	Receptor/Pathway	References
Alum	Aluminum Salt	NLRP3, DNA	(48)
Matrix-M/IscoMatrix	Saponin	Unknown	(49)
MF59	Squalene oil, surfactant	IL-4, STAT6	(50)
AS03	Squalene oil, surfactant, α-tocopherol	G-CSF	(48)
CPG1018	Synthetic DNA alone or formulated with Alum	TLR9	(48)
Ligand adsorbed in Alum	Ligand adsorbed in Alum	TLR7/TLR8	(48)

on existing adjuvants used in COVID-19 vaccines. Alum is widely employed as an adjuvant in vaccine development across the globe. It facilitates the production of antibodies and specific CD4+ T cells, albeit at low levels, by promoting anti-phagocytosis and activating the proinflammatory NLRP3 pathway. Moreover, Aluminum adjuvants are renowned for reducing immune-related pathological reactions and enhancing the safety of vaccines. This characteristic explains why BBIBP-CorV and CoronaVac vaccines, which utilize Aluminum hydroxide as adjuvants, are considered safe (44).

While the immunogenicity of aluminum adjuvant is poor, it can be significantly enhanced through chemical modification with short peptide antigens composed of repeated serine phosphate residues, thereby boosting GC cells and antibody responses. MF59, a squalene oil-in-water emulsion adjuvant, has gained approval for use in influenza vaccines in over 38 countries. It is noteworthy for being both biodegradable and biocompatible (45). The MF59 adjuvant has exhibited excellent tolerance and safety profiles. When administered in vaccine injections, it can activate macrophages and stimulate chemokine production. Additionally, MF59 stimulates the IL-4 and STAT6 signal pathways and induces an antibody response. Importantly, this response does not rely on IFN-I or inflammatory pathways. These favorable attributes have led to MF59 being selected as the adjuvant for COVID-19 vaccines (46).

AS03, a close relative of MF59, possesses unique features that distinguish it from its counterpart. It elicits a transient innate immune response, as evidenced by the transient production of cytokines observed in a mouse model upon injection. Furthermore, AS03 incorporates an additional immune-enhancing component: α-tocopherol (vitamin E). This combination has been evaluated as the adjuvant for several recombinant S protein vaccines in clinical trials. The inclusion of AS03 has been demonstrated to further enhance Th2-unbiased cell responses and the production of IFN-γ, potentially boosting the efficacy of COVID-19 vaccines (44).

CoVaccine HT is an oil-in-water emulsion, while CpG is a synthetic DNA sequence with an unmethylated CpG sequence. Both AMP-CpG and CoVaccine HT have demonstrated higher immunogenicity than the aluminum hydroxide adjuvant. The AMP-CpG adjuvant-induced persistent antibodies and T-cell reactions in elderly mice, even at low S protein doses, potentially improve vaccine safety. CoVaccine HT can elicit a quicker immune response to SARS-CoV-2 than aluminum hydroxide by promoting neutralizing antibody production and maturation. The use of aluminum adjuvants in vaccines may improve safety by reducing adverse events, and their poor immunogenicity can be addressed through the use of different adjuvants while ensuring the safety of subjects (44, 46).

A state-of-the-art vaccine for SARS-CoV-2 has been developed using Coronavirus-like particles (CoVLP) derived from plants and paired with the Adjuvant System 03 (AS03). These CoVLPs incorporate the prefusion spike glycoprotein from the initial strain of SARS-CoV-2. The CoVLP+AS03 vaccine has demonstrated efficacy in preventing COVID-19 caused by various strains, with efficacy rates ranging from 69.5% against symptomatic infection to 78.8% against moderate-to-severe disease (47). Table 1 presents an overview of adjuvants used in COVID-19 vaccines, along with their components and associated receptors/pathways.

Limitation of Novel Adjuvants

Although novel adjuvants have been developed to improve vaccine performance, they present several limitations that must be carefully considered. Foremost among these are safety concerns. The introduction of novel adjuvants can lead to unexpected immune reactions, including inflammatory responses or allergic reactions.

These adverse effects may undermine public confidence in vaccination programs. The rigorous and extensive testing required to establish safety profiles can hinder the timely deployment of promising adjuvants, particularly in response to emerging infectious diseases (51). Recent reports indicate a possible connection between the occurrence of vasculitis and autoimmune syndromes in individuals receiving COVID-19 vaccines, which could be due to an "adjuvant" in the vaccines. Studies have shown that autoimmune diseases, such as SLE, and infections can elevate age-associated B cell (ABC) levels in the body. These cells generate immunoglobulin G, enhance antigen presentation to T cells, and facilitate the formation of germinal centers. Moreover, through TLR7 signaling, ABC cells elicit a heightened response, which may produce auto-reactive antibody-secreting plasma blasts. The mRNA/DNA vaccines for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) use TLR7, TLR8, and TLR9 agonists as adjuvants, which may cause post-vaccine autoimmune syndromes by stimulating the ABC subgroup to develop autoantibodies. The activation of TLR7 and TLR9 can produce IFN-I, an essential cytokine in the development of SLE and other ARDs (52). However, the specific mechanisms by which post-COVID-19 vaccine autoimmune syndromes occur have yet to be determined.

Furthermore, the mechanisms of action of many new adjuvants remain poorly understood. This lack of comprehensive knowledge can complicate the selection of appropriate adjuvants for specific vaccines. Without a clear understanding of how an adjuvant interacts with the immune system, the potential for suboptimal immune responses increases, thereby jeopardizing the overall effectiveness of the vaccine (51). In addition, the cost of developing and producing new adjuvants can be prohibitive. Financial constraints may limit their incorporation into national vaccination programs, particularly in low-resource settings. This disparity can exacerbate global health inequities by rendering some populations unable to access the latest advancements in vaccine technology (7, 51). Moreover, regulatory pathways for new adjuvants can be complex and time-consuming. The necessity for extensive preclinical and clinical testing can delay the introduction of new adjuvants into the market, which is particularly critical during public health emergencies (51). In conclusion, while new adjuvants hold significant promise for enhancing vaccine efficacy, their limitations—including safety concerns, unclear mechanisms of action, high development costs, and complex regulatory requirements—pose formidable challenges. Addressing these limitations is imperative to fully realize the potential of adjuvants in improving global health outcomes through vaccination.

Conclusion

The primary aim of immunization is to deliver adequate and enduring protection against various infectious diseases. This immune defense can be achieved only through vaccine formulations incorporating appropriate adjuvants and antigens. Adjuvants play a crucial role in vaccine formulations, as they have the capacity to influence the immune response elicited by the vaccine. Despite the necessity of adjuvants, it is imperative to consider their safety and potential toxicity. As discussed in this article, evidence suggests that the use of adjuvants may lead to various autoimmune diseases in certain individuals. Furthermore, the safety of these substances in pediatric vaccinations appears to be a matter of concern. Nevertheless, the application of practical and suitable adjuvants in preventive vaccines, such as cancer vaccines, can significantly contribute to saving lives. As previously mentioned, adjuvants like BCG can prevent numerous immune-related diseases, which counters the notion that vaccines are harmful. The development of new and safe

vaccines that stimulate all components of the immune system, along with the completion of various clinical trials, is essential. The COVID-19 pandemic has once again drawn global attention to the safety and efficacy of vaccines and adjuvants.

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Conflict of interest

The authors declare that they have no conflicts of interest.

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Ethical Considerations

As this study is a review, it does not involve any ethical considerations.

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