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The Importance and Use of Adjuvants in Vaccine Production Technology: A Mini-review

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Authors' contributions

This work was carried out in collaboration among all authors. The authors MJMdP, EdOL, FdJO, AOdSS, and TTdS wrote and drafted the manuscript, as well as performed the translation to English. The remaining authors managed the study analyses and conducted the revisions and literature searches. All authors read and approved the final manuscript.

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Minireview Article

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ABSTRACT

Aims: To provide an overview of vaccine adjuvants, with insights into the importance, classification, and use of these substances in vaccine production technology.
Methodology: An exploratory-descriptive literature review was carried out, with a qualitative approach. The search was guided by keywords (vaccine adjuvant, chemical composition of vaccine, immunological adjuvants, aluminum salts + vaccine, among others) and was conducted according to the following criteria: original studies published during the period between 2000 and 2024, and available as full text; those using experimental and clinical studies as methodology were included.
Results: Vaccine adjuvants play an important role in the success of the vaccine technology used. With the advancement of knowledge, adjuvants have gone from substances used to increase the immunogenicity of vaccines to highly purified antigen substances that induce a response, acting as molecular patterns associated with pathogens. In this study, the most common classes of adjuvants in use or experimental studies, their characteristics, benefits, and limitations of use are presented. There are classes of adjuvants that are already well known in terms of their use and effects (e.g.: mineral salts). However, there are also those (e.g. polysaccharides) that require even more studies to be widely incorporated into vaccine technology.
Conclusion: Adjuvants are an integral part of the ongoing development of more effective vaccines. Therefore, it is necessary to continue studies regarding the benefits and limitations of the different types of adjuvants currently available, such as continuing to search for new adjuvants to expand and increasingly guarantee the success of vaccine technologies.

Keywords: Immunization; chitosan; mineral salts; immune system; vaccinology.

1. INTRODUCTION

The vaccination technique can be seen as an ancient practice, present since the 900s A.D. known as variolation, the method developed in Chinese culture involved the application of dried pus to healthy patients. However, although effective in inducing immunity, the practice often leads to the development of the disease in a severe form, resulting in a high percentage of mortality [1]. It was Edward Jenner who in 1976 demonstrated a safe method for immunization through cowpox, thus creating the first vaccine in 1978 [2].

After Jenner's discoveries, Louis Pasteur invented the next advance in the development of vaccines, which was based on the principle of attenuation. In 1880, nearly a century after Edward Jenner's experiments, Pasteur succeeded in producing a vaccine against cholera in chickens caused by the bacteria *Pasteurella multocida* [3]. These and other studies culminated in the development of traditional vaccine technologies, which are still used today, and are based on killed/inactivated and live/attenuated approaches [4].

Over the years, vaccinology has advanced a lot and, as a result, other technologies have been developed and added to previously existing options, based on dead or attenuated microorganisms. This is the case of multivalent subunit vaccine technologies, DNA vaccines, recombinant vector vaccines or mRNA [5-7]. These advances in vaccine technology have contributed to vaccination becoming one of the most successful examples of public health globally and acts to prevent disease through the induction of immunization by pathogen antigens [8-10].

In addition to this classification by development technology, vaccines can also be classified into generations. For example, vaccines developed with live-attenuated or inactivated pathogens, the first vaccine technology developed, are classified as first-generation. On the other hand, multivalent subunit vaccines and recombinant vaccines are considered second-generation and DNA vaccines are considered third-generation

[4]. Although it is a technology that has been studied for a long time [11], mRNA vaccines have only been used on a large scale in recent years, in the fight against the COVID-19 pandemic [6,12,13], also being considered third-generation.

It is a fact that the antigens present in vaccines are responsible for inducing the immune response. However, vaccines can also be made up of other elements, such as adjuvants, stabilizers, and preservatives [14]. Furthermore, they may also contain traces of particles used in their production such as antibiotics, egg protein, or formaldehyde [15]. These substances play an important role in vaccine technology to enable adequate immunization.

Initially, the class of adjuvants was used to increase the immunogenicity of highly purified antigen vaccines. Several of them used in industry were discovered empirically [16-18]. As knowledge about vaccine technology advances, many adjuvants are developed to induce a response by acting as pathogen-associated molecular patterns - PAMPs [1].

Stabilizers provide properties to increase the thermostable characteristics of vaccines and are made up of buffers, proteins, antioxidants, amino acids, sugars, and surfactants [19]. The choice of stabilizers is related to the vaccine technology and the active pharmaceutical ingredient. The class of preservatives is associated with the production and storage process and aims to reduce or avoid contamination by microorganisms. One of the main preservative agents is 2-phenoxyethanol [14]. These, together with stabilizers, form the excipient of vaccine formulations.

In light of all this, to expand knowledge about vaccines and their components, and contribute to the end of misinformation on the topic, this mini-review aims to provide an overview of vaccine adjuvants, with insights into the importance, classification, and use of these substances in vaccine production technology.

2. METHODOLOGY

An exploratory-descriptive literature review was carried out, with a qualitative approach. The search was guided by keywords (vaccine adjuvant, chemical composition of vaccine, immunological adjuvants, aluminum salts + vaccine, among others) and was conducted according to the following criteria: original studies published in 2000-2024, and available as full text; those using experimental and clinical studies as methodology were included (24 studies). Studies published in conferences or presentations and those not directly contributing to the researched topic were excluded (40 studies). The main databases used were Scientific Electronic Library Online (SciELO), PUBMED, and Scopus.

3. RESULTS AND DISCUSSION

3.1 Vaccinology: Historical Aspects

The first vaccines were developed by Edward Jenner in 1796, after 20 years of studies and experiments with cowpox [20]. Jenner discovered that protection against smallpox could be achieved by inoculating material extracted from cowpox pustular lesions into humans. The material that was inoculated became known as “vaccine”, which is derived from the Latin term “Vacca”, which means cow, and the process was called "vaccination" [21].

Over the years, scientific advances have contributed to understanding the effects of vaccine adjuvants, substances added to vaccines to reinforce the immune response. French veterinarian Gaston Ramon proved, in his studies with equine anti-tetanus and diphtheria antisera, that substances capable of inducing inflammation at the injection site could also increase the effectiveness of antisera [22].

During this same period, Alexander Glenny discovered the immune-boosting effects of aluminum salts. In 1932, aluminum was used for the first time as an adjuvant in human vaccines and became the first licensed adjuvant in use [23]. Aluminum adjuvants increase antibody production and are ideal for pathogens killed by antibodies.

Adjuvants have been used for more than 90 years and are present in more than 30 licensed vaccines from different manufacturers. However, some attenuated vaccines are capable of inducing mild infections in recipients, activating an immune response similar to innate immunity. This eliminates the need for adjuvants in these vaccines. The safety of adjuvants is assessed according to the vaccine in which they are used [1].

Currently, the development of vaccines follows more stringent requirements, which require high

effectiveness, long duration of protection, and absence of adverse effects [24]. Scientific and technological advances have contributed to the continuous improvement of vaccines, which seek to meet public health demands efficiently and safely [25].

3.2 Classification and Use of Adjuvants Vaccines

3.2.1 Adjuvants derived from microorganisms

Adjuvants play a crucial role in vaccine development. Its ability to stimulate specific subsets of T cells, such as CD4+ TH1 and TH2 cells, as well as CD8+ cells involved in cytotoxic responses, is closely linked to adjuvanticity [26, 27].

Natural products, from plants, bacteria, or fungi, are potential sources of new vaccine adjuvants due to their powerful immunostimulatory capacity [28,29]. Although not highly immunogenic, peptidoglycan or LPS (lipopolysaccharide) from bacterial cell membranes enhance the immune response. This adjuvant activity occurs by activating toll-like receptors (TLRs), which signal danger and activate the host's immune system [30].

Bacterial species used as sources of adjuvants include *Mycobacterium* spp., *Corynebacterium parvum*, *C. granulosum*, *Bordetella pertussis*, and *Neisseria meningitidis*. However, these complete microorganisms are too toxic to be used as adjuvants in humans [30].

The lipophilic chain at the C-terminal end of the peptide increases the effectiveness of MDP, resulting in stronger specific immune responses. Furthermore, this lipophilic chain also increases nonspecific resistance against bacterial or parasitic infections under certain conditions [31]. LPS is a compound derived from the wall of Gram-negative bacteria, and its main adjuvant effect is attributed to lipid A. Under low acidity conditions, LPS can be hydrolyzed to obtain MPLA, a compound that retains the adjuvant activity of lipid A, reducing its toxicity [30]. TDM is an immunostimulant found on the surface of bacteria that promotes cell-mediated and humoral responses.

3.2.2 Water-in-oil emulsions

Small oil droplets stabilized by surfactants in a continuous aqueous phase characterize oil-in-water emulsions. Through the slow release of antigens, adjuvants in this category stimulate the immune system in a lasting way [32].

The first documented use of oil emulsions was in 1916, when Le Moignic and Pinoy vaccinated mice with Salmonella typhimurium inactivated and emulsified with mineral oil [33]. However, the frequent use of emulsions as adjuvants began in 1956, with the introduction of complete Freund's adjuvant (CFA), widely used in animal formulations but considered unacceptable for use in humans.

The first approved adjuvant emulsion was MF59, a squalene oil-based adjuvant that received initial authorization for use in the Fluad® vaccine in 1997. In one study, MF59 stimulated cellular recruitment in the muscles where it was injected, resulting in large quantities of neutrophils, granulocytes, monocytes, macrophages, and dendritic cells [34]. MF59 is licensed as an adjuvant in seasonal influenza vaccines for the elderly and pandemic influenza vaccines for H1N1 and H5N1. Furthermore, this adjuvant is being evaluated for use in vaccines against HIV, cytomegalovirus, and hepatitis C.

AS03 is another squalene-based oil-in-water emulsion adjuvant, similar to MF59, and is approved and licensed for global use. To date, the primary use of the AS03 adjuvant is in Pandemrix® pandemic influenza vaccines. Vaccines with the AS03 adjuvant have been demonstrated to be immunogenic, effective, and well-tolerated, with acceptable safety profiles [35].

Water and oil emulsions are considered safe and have low production costs, making them excellent adjuvants in vaccine formulation. Its safety is attributed to the low proportion of oil used, generally in the range of 15% to 25%. However, these adjuvants can be unstable if the oil used is not completely purified and does not have the ability to alter the action of cytokines, resulting in weak immunomodulation [36].

3.2.3 Polysaccharides

Due to the rapid advancement of material science, biomaterials are being developed for a variety of applications. Biopolymers emerge as promising candidates to replace existing materials due to their ease of synthesis in different forms, good mechanical properties, biocompatibility, and biodegradability. The use of

biopolymers as adjuvants has demonstrated effectiveness in activating the immune response, promoting the circulation of antigens, and reducing the need for booster doses [37].

In the 1980s, it was discovered that xanthan gum had intrinsic activity as an adjuvant, acting as a lymphocyte activator. Currently, xanthan gum is used in the formulation of nasal vaccines against influenza, achieving success as an adjuvant [38]. Studies have also shown the potential of xanthan gum as an adjuvant in vaccines against Leptospirosis.

Recent studies have explored the use of inulin, a polysaccharide found in the roots of plants in the Compositae family, which belongs to one of the largest families of dicotyledonous angiosperms. Inulin can be found in four forms, distinguished by solubility: alpha, gamma, beta, and delta. The gamma and delta forms have stood out for their adjuvant activity, stimulating a robust cellular and humoral immune response, without presenting toxicity [39].

Researchers at Petrovsky (Flinders) conducted studies to investigate the adjuvant activities of delta inulin against several infectious pathogens. The delta form of inulin was found to have a stronger immune-boosting effect than the gamma form in a study with hepatitis B antigens. Based on this finding and other studies with the delta form of inulin, the adjuvant was developed Advax™, formulated from this specific form of inulin. Advax™ adjuvants have demonstrated efficacy in several vaccines, proven through studies in animal models and clinical trials in humans [40].

Chitosan has also been reported as a vaccine adjuvant and is obtained through alkaline partial deacetylation of chitin [41-43]. The mucoadhesive, biocompatibility, biodegradability, and less toxic properties of chitosan compared to the other vaccine adjuvants made it a promising candidate for use as an adjuvant in vaccine technology [44]. The immunostimulatory properties of this polymer, which make it interesting as a vaccine adjuvant, have been attributed to several reasons, which may be related to the induction of humoral responses and cell-mediated immune responses in experimental models [44,45].

Studies have shown that chitosan is capable of inducing immunity when used in the form of microparticles. In vitro assays indicated that encapsulated chitosan particles stimulate macrophage activation. Furthermore, in vivo assays with powdered chitosan nanospheres encapsulated with influenza virus demonstrated induction of humoral response and cellular immune responses after nasal administration in rabbits [46].

3.2.4 Mineral salts

Potassium aluminum sulfate (alum) was the first mineral adjuvant discovered in 1926 by Glenny. Since then, several derivatives of this compound have been used in several vaccines. Among the adjuvants derived from alum, aluminum phosphate, aluminum hydroxide, aluminum oxyhydroxide, and aluminum hydroxyphosphate sulfate stand out [15]. In addition to these aluminum compounds, calcium phosphate, a compound discovered by Edgar Relyvel in 1958, is also used as an adjuvant [47].

These salts are classified as vehicles or immunostimulants, depending on their mechanism of action [15]. In Table 1, some vaccines and their respective mineral salt adjuvants are listed.

Table 1. Examples of vaccines whose adjuvants are mineral salts

Vaccine	Adjuvant
HPV (Cervarix)	Aluminum hydroxide
Pneumococcal conjugate vaccine (Prevnar and Synflorix)	Aluminum Phosphate
Hepatitis B (Engerix B)	Aluminum hydroxide
Hepatitis B (Recombivax HB)	Aluminum hydroxyphosphate Sulfate
HPV (Gardasil)	Aluminum hydroxyphosphate
Porcine deltacoronavirus	Aluminum hydroxide

Table 2. Examples of vaccines whose adjuvants are surfactants

Vaccine	Adjuvant
Influenza A	SF-10
Carbuncle	Span-60 + cholesterol and aluminum hydroxide
Influenza A	Quil-A + cGAMP

3.2.5 Surfactant components (surfactants)

Surfactant components or surfactants are amphiphilic compounds classified as non-ionic, anionic, cationic, or amphoteric [48]. Vaccine prototypes with this type of adjuvant are mentioned in the literature, some are even used in combination with mineral salts [14,49,50]. Table 2 lists some vaccines and their respective surfactant-type adjuvants.

The SF-10 is a synthetic mucosal adjuvant derived from physiological metabolic pathways and function of human pulmonary surfactant. This adjuvant was shown to be effective as a mucosal adjuvant that is required for vaccination against influenza A virus (IAV) infection [50]. This adjuvant is similar to Surfacten®, a bovine component that demonstrated efficiency as an intranasal mucosal adjuvant combined with inactivated influenza split vaccine, induced systemic IgG and mucosal S-IgA [51]. Contudo, o component SF-10 é synthetic mucosal adjuvant derived from pulmonary surfactant, which does not contain any bovine component [52].

Surfactant components can also be used in combination with other classes of adjuvants. For example, Span-60, a nonionic surfactant, in combination with cholesterol and aluminum hydroxide was a combination proposed by Gogoi et al. [14] to achieve enhanced Immune response and superior protection against Anthrax. In this case, the authors studied the synergistic effect of aluminum hydroxide nanoparticles (AH np) and non-ionic surfactant-based vesicles (NISV), which was prepared with Span 60 and cholesterol, in modulating the immune response against protective antigen domain 4 (D4) of *Bacillus anthracis*. The combination was shown to be able to increase the humoral and cellular response, therefore being a very promising result.

Like other classes of adjuvants, surfactants still require further studies so that they can be increasingly used safely and efficiently in vaccines, as has been done for SF-10 [50] and the combination of Span-60 + cholesterol and aluminum hydroxide [14].

4. CONCLUSION

Adjuvants are an integral part of the ongoing development of more effective vaccines. Therefore, it is necessary to continue studies regarding the benefits and limitations of the different types of adjuvants currently available, such as continuing to search for new adjuvants to expand and increasingly guarantee the success of vaccine technologies.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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