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

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

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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 1776 demonstrated a safe method for immunization through cowpox, thus creating the first vaccine in 1789 [2].

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

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

37 In addition to this classification by development technology, vaccines can also be classified
38 into generations. For example, vaccines developed with live-attenuated or inactivated
39 pathogens, the first vaccine technology developed, are classified as first-generation. On the
40 other hand, multivalent subunit vaccines and recombinant vaccines are considered second-
41 generation and DNA vaccines are considered third-generation [4]. Although it is a technology
42 that has been studied for a long time [11], mRNA vaccines have only been used on a large
43 scale **in recent years**, in the fight against the COVID-19 pandemic [6, 12, 13], also being
44 considered third-generation.

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

50 Initially, the class of adjuvants was used to increase the immunogenicity of highly purified
51 antigen vaccines. Several of them used in industry were discovered empirically[50-52]. As
52 knowledge about vaccine technology advances, many adjuvants are developed to induce a
53 response by acting as pathogen-associated molecular patterns - PAMPs [1].

54 Stabilizers provide properties to increase the thermostable characteristics of vaccines and
55 are made up of buffers, proteins, antioxidants, amino acids, sugars, and surfactants [16].
56 The choice of stabilizers is related to the vaccine technology and the active pharmaceutical
57 ingredient. The class of preservatives is associated with the production and storage process
58 and aims to reduce or avoid contamination by microorganisms. One of the main preservative
59 agents is 2-phenoxyethanol [14]. These, together with stabilizers, form the excipient of
60 vaccine formulations.

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

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67 2. METHODOLOGY

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69 An exploratory-descriptive literature review was carried out, with a qualitative approach. The
70 search was guided by keywords (vaccine adjuvant, chemical composition of vaccine,

71 immunological adjuvants, aluminum salts + vaccine, among others) and was conducted
72 according to the following criteria: original studies published in 2000-2024, and available as
73 full text; those using experimental and clinical studies as methodology were included (24
74 studies). Studies published in conferences or presentations and those not directly
75 contributing to the researched topic were excluded (40 studies). The main databases used
76 were Scientific Electronic Library Online (SciELO), PUBMED, and Scopus.

77

78 **3. RESULTS AND DISCUSSION**

79

80 **3.1 Vaccinology: historical aspects**

81 The first vaccines were developed by Edward Jenner in 1796, after 20 years of studies and
82 experiments with cowpox [17]. Jenner discovered that protection against smallpox could be
83 achieved by inoculating material extracted from cowpox pustular lesions into humans. The
84 material that was inoculated became known as “vaccine”, which is derived from the Latin
85 term “Vacca”, which means cow, and the process was called "vaccination" [18].

86 Over the years, scientific advances have contributed to understanding the effects of vaccine
87 adjuvants, substances added to vaccines to reinforce the immune response. French
88 veterinarian Gaston Ramon proved, in his studies with equine anti-tetanus and diphtheria
89 antisera, that substances capable of inducing inflammation at the injection site could also
90 increase the effectiveness of antisera [19].

91 During this same period, Alexander Glennie discovered the immune-boosting effects of
92 aluminum salts. In 1932, aluminum was used for the first time as an adjuvant in human
93 vaccines and became the first licensed adjuvant in use [20]. Aluminum adjuvants increase
94 antibody production and are ideal for pathogens killed by antibodies.

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

100 Currently, the development of vaccines follows more stringent requirements, which require
101 high effectiveness, long duration of protection, and absence of adverse effects [21].
102 Scientific and technological advances have contributed to the continuous improvement of
103 vaccines, which seek to meet public health demands efficiently and safely [22].

104

105 **3.2 Classification and use of adjuvants vaccines**

106 3.2.1 Adjuvants derived from microorganisms

107 Adjuvants play a crucial role in vaccine development. Its ability to stimulate specific subsets
108 of T cells, such as CD4+ TH1 and TH2 cells, as well as CD8+ cells involved in cytotoxic
109 responses, is closely linked to adjuvanticity [23, 24].

110 Natural products, from plants, bacteria, or fungi, are potential sources of new vaccine
111 adjuvants due to their powerful immunostimulatory capacity [25, 26]. Although not highly
112 immunogenic, peptidoglycan or LPS (lipopolysaccharide) from bacterial cell membranes

113 enhance the immune response. This adjuvant activity occurs by activating toll-like receptors
114 (TLRs), which signal danger and activate the host's immune system [27].

115 Bacterial species used as sources of adjuvants include *Mycobacterium* spp.,
116 *Corynebacterium parvum*, *C. granulorum*, *Bordetella pertussis*, and *Neisseria meningitidis*.
117 However, these complete microorganisms are too toxic to be used as adjuvants in humans
118 [27].

119 The lipophilic chain at the C-terminal end of the peptide increases the effectiveness of MDP,
120 resulting in stronger specific immune responses. Furthermore, this lipophilic chain also
121 increases nonspecific resistance against bacterial or parasitic infections under certain
122 conditions [28]. LPS is a compound derived from the wall of Gram-negative bacteria, and its
123 main adjuvant effect is attributed to lipid A. Under low acidity conditions, LPS can be
124 hydrolyzed to obtain MPLA, a compound that retains the adjuvant activity of lipid A, reducing
125 its toxicity [27]. TDM is an immunostimulant found on the surface of bacteria that promotes
126 cell-mediated and humoral responses.

127

128 3.2.2 Water-in-oil emulsions

129 Small oil droplets stabilized by surfactants in a continuous aqueous phase characterize oil-
130 in-water emulsions. Through the slow release of antigens, adjuvants in this category
131 stimulate the immune system in a lasting way [29].

132 The first documented use of oil emulsions was in 1916, when Le Moignic and Pinoy
133 vaccinated mice with *Salmonella typhimurium* inactivated and emulsified with mineral oil
134 [30]. However, the frequent use of emulsions as adjuvants began in 1956, with the
135 introduction of complete Freund's adjuvant (CFA), widely used in animal formulations but
136 considered unacceptable for use in humans.

137 The first approved adjuvant emulsion was MF59, a squalene oil-based adjuvant that
138 received initial authorization for use in the Fluvad® vaccine in 1997. In one study, MF59
139 stimulated cellular recruitment in the muscles where it was injected, resulting in large
140 quantities of neutrophils, granulocytes, monocytes, macrophages, and dendritic cells [31].
141 MF59 is licensed as an adjuvant in seasonal influenza vaccines for the elderly and pandemic
142 influenza vaccines for H1N1 and H5N1. Furthermore, this adjuvant is being evaluated for
143 use in vaccines against HIV, cytomegalovirus, and hepatitis C.

144 AS03 is another squalene-based oil-in-water emulsion adjuvant, similar to MF59, and is
145 approved and licensed for global use. To date, the primary use of the AS03 adjuvant is in
146 Pandemrix® pandemic influenza vaccines. Vaccines with the AS03 adjuvant have been
147 demonstrated to be immunogenic, effective, and well-tolerated, with acceptable safety
148 profiles [32].

149 Water and oil emulsions are considered safe and have low production costs, making them
150 excellent adjuvants in vaccine formulation. Its safety is attributed to the low proportion of oil
151 used, generally in the range of 15% to 25%. However, these adjuvants can be unstable if the
152 oil used is not completely purified and does not have the ability to alter the action of
153 cytokines, resulting in weak immunomodulation [33].

154 3.2.3 Polysaccharides

155 Due to the rapid advancement of material science, biomaterials are being developed for a
156 variety of applications. Biopolymers emerge as promising candidates to replace existing
157 materials due to their ease of synthesis in different forms, good mechanical properties,
158 biocompatibility, and biodegradability. The use of biopolymers as adjuvants has
159 demonstrated effectiveness in activating the immune response, promoting the circulation of
160 antigens, and reducing the need for booster doses [34].

161 In the 1980s, it was discovered that xanthan gum had intrinsic activity as an adjuvant, acting
162 as a lymphocyte activator. Currently, xanthan gum is used in the formulation of nasal
163 vaccines against influenza, achieving success as an adjuvant [35]. Studies have also shown
164 the potential of xanthan gum as an adjuvant in vaccines against Leptospirosis.

165 Recent studies have explored the use of inulin, a polysaccharide found in the roots of plants
166 in the Compositae family, which belongs to one of the largest families of dicotyledonous
167 angiosperms. Inulin can be found in four forms, distinguished by solubility: alpha, gamma,
168 beta, and delta. The gamma and delta forms have stood out for their adjuvant activity,
169 stimulating a robust cellular and humoral immune response, without presenting toxicity [36].

170 Researchers at Petrovsky (Flinders) conducted studies to investigate the adjuvant activities
171 of delta inulin against several infectious pathogens. The delta form of inulin was found to
172 have a stronger immune-boosting effect than the gamma form in a study with hepatitis B
173 antigens. Based on this finding and other studies with the delta form of inulin, the adjuvant
174 was developed Advax™, formulated from this specific form of inulin. Advax™ adjuvants
175 have demonstrated efficacy in several vaccines, proven through studies in animal models
176 and clinical trials in humans [37].

177 Chitosan has also been reported as a vaccine adjuvant and is obtained through alkaline
178 partial deacetylation of chitin [38-40]. The mucoadhesive, biocompatibility, biodegradability,
179 and less toxic properties of chitosan compared to the other vaccine adjuvants made it a
180 promising candidate for use as an adjuvant in vaccine technology [41]. The
181 immunostimulatory properties of this polymer, which make it interesting as a vaccine
182 adjuvant, have been attributed to several reasons, which may be related to the induction of
183 humoral responses and cell-mediated immune responses in experimental models [41, 42].

184 Studies have shown that chitosan is capable of inducing immunity when used in the form of
185 microparticles. In vitro assays indicated that encapsulated chitosan particles stimulate
186 macrophage activation. Furthermore, in vivo assays with powdered chitosan nanospheres
187 encapsulated with influenza virus demonstrated induction of humoral response and cellular
188 immune responses after nasal administration in rabbits [43].

189

190 3.2.4 Mineral salts

191 Potassium aluminum sulfate (alum) was the first mineral adjuvant discovered in 1926 by
192 Glenny. Since then, several derivatives of this compound have been used in several
193 vaccines. Among the adjuvants derived from alum, aluminum phosphate, aluminum
194 hydroxide, aluminum oxyhydroxide, and aluminum hydroxyphosphate sulfate stand out [15].
195 In addition to these aluminum compounds, calcium phosphate, a compound discovered by
196 Edgar Relyvel in 1958, is also used as an adjuvant [44].

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

200 **Table 1. Examples of vaccines whose adjuvants are mineral salts.**

Vaccine	Adjuvant
HPV (Cervarix)	Aluminum hydroxide
Pneumococcal conjugate vaccine (Pevnar 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

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202 3.2.5 Surfactant components (surfactants)

203 Surfactant components or surfactants are amphiphilic compounds classified as non-ionic,
204 anionic, cationic, or amphoteric [45]. Vaccine prototypes with this type of adjuvant are
205 mentioned in the literature, some are even used in combination with mineral salts [14, 46,
206 47]. Table 2 lists some vaccines and their respective surfactant-type adjuvants.

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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

210

211 The SF-10 is a synthetic mucosal adjuvant derived from physiological metabolic pathways
212 and function of human pulmonary surfactant. This adjuvant was shown to be effective as a
213 mucosal adjuvant that is required for vaccination against influenza A virus (IAV) infection
214 [47]. This adjuvant is similar to Surfacten®, a bovine component that demonstrated
215 efficiency as an intranasal mucosal adjuvant combined with inactivated influenza split
216 vaccine, induced systemic IgG and mucosal S-IgA [48]. Contudo, o componente SF-10 é
217 synthetic mucosal adjuvant derived from pulmonary surfactant, which does not contain any
218 bovine component [49].

219 Surfactant components can also be used in combination with other classes of adjuvants. For
220 example, Span-60, a nonionic surfactant, in combination with cholesterol and aluminum

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

228 Like other classes of adjuvants, surfactants still require further studies so that they can be
229 increasingly used safely and efficiently in vaccines, as has been done for SF-10 [47] and the
230 combination of Span-60 + cholesterol and aluminum hydroxide [14].

231

232 4. Conclusion

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

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247 AUTHORS' CONTRIBUTIONS

248

249 This work was carried out in collaboration among all authors. The authors M.J.M. Paiva,
250 E.O. Lara, F.J. Oliveira, A.O.S. Sampaio, and T.T.Santos wrote and drafted the manuscript,
251 as well as performed the translation to English. The remaining authors managed the study
252 analyses and conducted the revisions and literature searches. All authors read and
253 approved the final manuscript.

254

255 Disclaimer (Artificial intelligence)

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257 Author(s) hereby declare that NO generative AI technologies such as Large Language
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264 the name, version, model, and source of the generative AI technology and as well as the all
265 input prompts provided to a generative AI technology

266

267 Details of the AI usage are given below:

268 1.

269 2.
270 3.
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