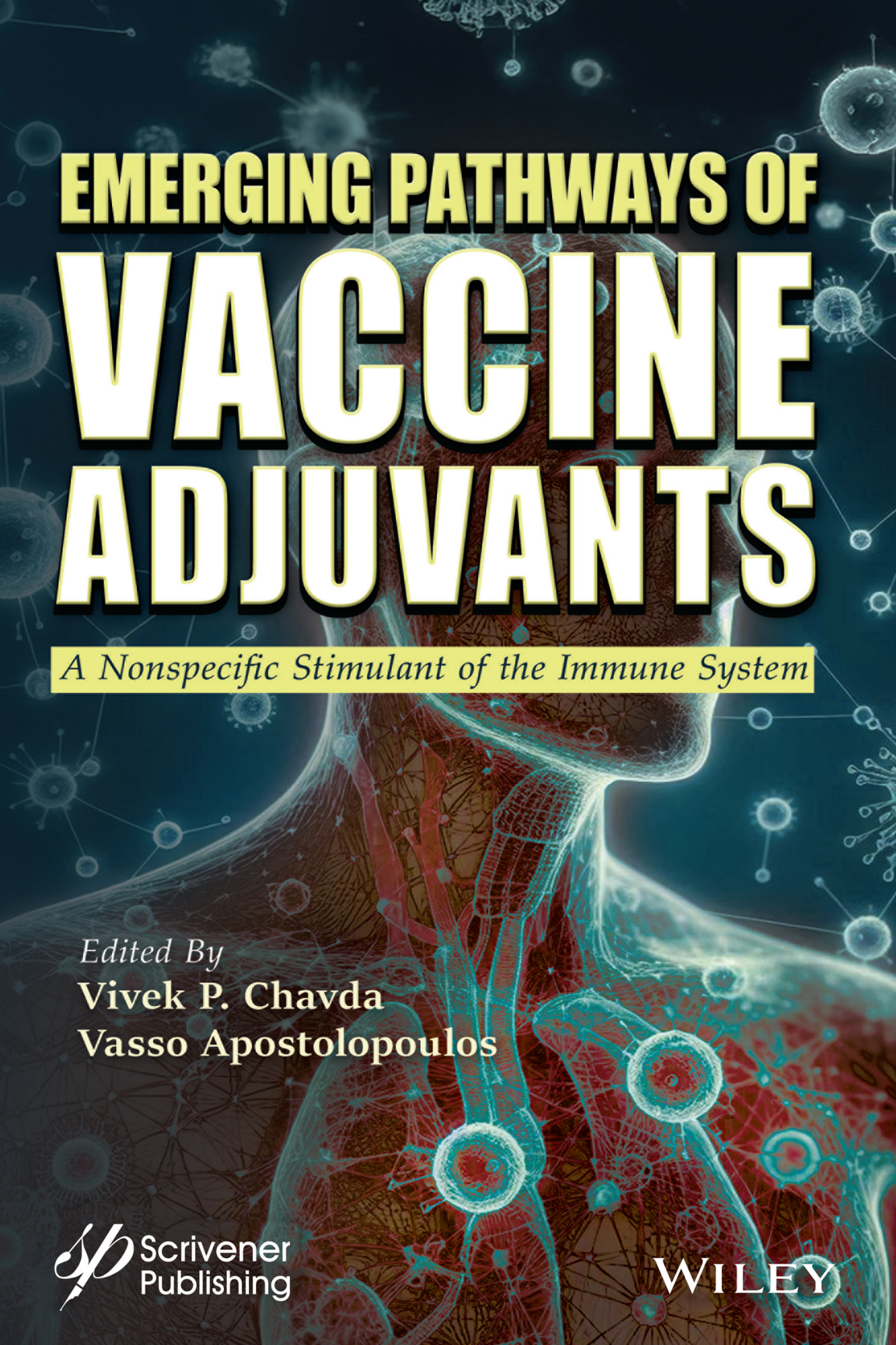
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A Nonspecific Stimulant of the Immune System
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A Nonspecific Stimulant of the Immune System

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Preface

Adjuvants play a crucial role in vaccine formulations by boosting the immunogenicity of antigens, thereby enhancing vaccine efficacy. While antigens can initiate immune responses independently, adjuvants amplify these responses. They do so by stimulating antigen-presenting cells and facilitating the maturation of these cells to effectively present antigenic peptides to both T and B cells. The main objective of this book is to provide readers with an in-depth understanding of the latest advancements in adjuvant technology. Ultimately, the book aims to drive progress in vaccine research, paving the way for the development of more potent and safer vaccines to address global health threats.

This book provides a comprehensive overview of the evolving landscape of vaccine adjuvants, encompassing a wide range of topics critical to their design, development, and application. Chapter 1 is an introductory chapter on adjuvants and Chapter 2 presents the cutting-edge field of *in silico* adjuvant design and validation, shedding light on computational approaches to optimize adjuvant properties. The chapter explores the intricate relationship between adjuvants and immunity (Chapter 3), elucidating how these immunomodulators enhance vaccine responses. Novel formulation strategies for vaccines incorporating adjuvants are discussed (Chapter 4), along with detailed characterization methods to ensure their quality and performance. The book also highlights the role of adjuvants in licensed vaccines (Chapter 5), emphasizing their contribution to vaccine efficacy. Emerging nanomaterial-based adjuvants (Chapter 6) and innovative non-invasive routes of vaccine delivery (Chapter 7) are explored as promising avenues for future vaccine development. Regulatory guidelines governing vaccine adjuvants are outlined to navigate the complex landscape of vaccine approval and licensing (Chapter 8). Importantly, the book addresses vaccine safety concerns associated with adjuvants (Chapter 9), discussing strategies to mitigate risks.

Chapter 10 highlights the limitations of adjuvants and explores future directions to advance the field of vaccinology.

The Editors
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November 2024

1

Adjuvants Boosting Vaccine Effectiveness

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Abstract

Vaccine development has evolved significantly with the identification and isolation of specific antigens, leading to subunit vaccines. Adjuvants, crucial in modern vaccine design, enhance antigen immunogenicity, allowing for more effective vaccines that stimulate both humoral and cell-mediated immunity. Conventional adjuvants, including aluminum salts, SAF-1, QS-21, and squalene-based adjuvants such as MF59 and AS03, play pivotal roles in enhancing vaccine efficacy. Particulate adjuvants, including liposomes, immunostimulatory complexes, and emulsions like MF59 and AS03, offer improved antigen stability and targeted delivery. Additionally, immunostimulatory adjuvants like Toll-like receptor agonists, monophosphoryl lipid A, cytokines, and CpG oligodeoxynucleotides directly activate immune responses. Approved adjuvants, AS01, AS03, AS04, MF59, Matrix-M, and virosomes are key adjuvants in approved human vaccines, enhancing immune responses and vaccine efficacy. Despite advancements, ongoing research is required to optimize adjuvant safety and efficacy in order to develop safer and more effective vaccines against infectious diseases and cancers.

Keywords: Adjuvants, vaccination, AS01, MF59, Matrix-M, virosomes, SAF-1, QS-21

1.1 Vaccines Over the Years

The history of vaccination spans over a millennium, with early attempts to prevent infectious diseases dating back to 1000 A.D. in China, where smallpox vesicles were used for inoculation. Edward Jenner's work in

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the late 1700s marked a significant advancement when he observed that individuals who had contracted cowpox were protected against smallpox. By 1796, Jenner successfully immunized a young boy with cowpox, confirming protection against smallpox. Louis Pasteur furthered the field by demonstrating the use of attenuated pathogens as vaccines in the late 19th century. He attenuated *Pasteurella septica* to develop a vaccine against fowl cholera and later applied a similar approach to *Bacillus* anthrax, achieving remarkable success in protecting farm animals. Additionally, Pasteur's work with rabies marked a significant milestone in the development of live virus vaccines. In the realm of dead organism vaccines, the Salk vaccine against poliomyelitis, developed in 1960, had a profound impact on disease incidence before being succeeded by the Sabin vaccine. Challenges persisted in producing killed vaccines due to potential destruction of important antigenic components.

The identification and isolation of specific antigens responsible for protection paved the way for "subunit" and "extract" vaccines. For instance, diphtheria and tetanus toxoids were purified and inactivated using formalin, retaining their antigenicity but reducing adverse reactions. Despite these advancements, the history of vaccine development is not without setbacks. Disasters such as the Lubeck Disaster in 1932, where infants were mistakenly given *Mycobacterium tuberculosis* instead of BCG vaccine, and the Cutter Disaster in 1955, where a faulty polio vaccine led to cases of poliomyelitis, highlighted the need for stringent quality control and safety measures. As public awareness and standards for vaccine safety have increased, modern vaccinology has embraced advancements in genetics, chemistry, peptide synthesis, protein production methods, DNA, mRNA, x-ray crystal structures, molecular biology, and immunology, allowing for the development of safer and more efficient vaccines [1]. However, there are still many obstacles for their clinical use, and the limited immunogenicity of many of these candidates has hindered their development as potential vaccines. Strategies to enhance the immunogenicity of candidate vaccines are therefore critical. As such, adjuvants have been developed to enhance immunogenicity of vaccines, aiming to overcome their limited efficacy. These advancements are critical for optimizing the clinical potential of novel vaccine candidates.

1.2 Adjuvants in the Modern Era

Adjuvants play a pivotal role in modern vaccine development, enhancing the immune response to antigens and thereby improving vaccine efficacy [2–4]. While antigens alone can stimulate the immune system to some

extent, adjuvants amplify this response, making vaccines more effective at inducing both humoral and cell-mediated immunity. This is particularly crucial for subunit vaccines, which consist of purified antigens and often require adjuvants to boost their immunogenicity. Additionally, adjuvants can help reduce the amount of antigen needed per dose, which is beneficial for both vaccine production and delivery. Adjuvants enable the use of novel vaccine technologies, such as synthetic peptides and recombinant proteins, which may otherwise lack sufficient immunogenicity to elicit a protective immune response [5]. Despite their importance, the development and use of adjuvants in human vaccines have been limited by safety concerns, requiring the need for rigorous testing and evaluation. As the area of vaccine development continues to advance, the discovery and optimization of safe and effective adjuvants remain a critical area of research, holding the potential to revolutionize vaccine design and contribute to global health by combating infectious diseases more effectively [6].

1.3 Conventional Adjuvants

Adjuvants play a crucial role in enhancing antigen immunogenicity, amplifying both humoral and cell-mediated immune responses. A widely used adjuvant in experimental animals is complete Freund's adjuvant (CFA), a water-in-oil emulsion containing killed *M. tuberculosis*. Despite its effectiveness and long sustained immune responses, CFA is not suitable for human use due to its propensity to induce granulomas, fever, and inflammation. Incomplete Freund's adjuvant, which lacks the mycobacterial component, has been evaluated, which does not induce granulomas and is safer than CFA, but it is still not approved for human vaccines due to other safety concerns. However, aluminum salts approved for human use in the 1930s, being either as aluminum hydroxide or aluminum phosphate, are the most widely used adjuvants in human vaccines. They enhance the immune response by forming a depot at the injection site, facilitating antigen uptake by antigen-presenting cells and stimulating cytokine secretion [7–9]. Alum primarily stimulates humoral immune responses and is used in vaccines against diphtheria, tetanus, and hepatitis B. However, aluminum-based adjuvants primarily stimulate humoral immune responses and are limited in cell-mediated immune stimulation (Figure 1.1). Emerging conventional adjuvants include the following:

a. **SAF-1:** Comprising squalene oil, threonyl-MDP, and non-ionic block polymers, SAF-1's block polymers act as

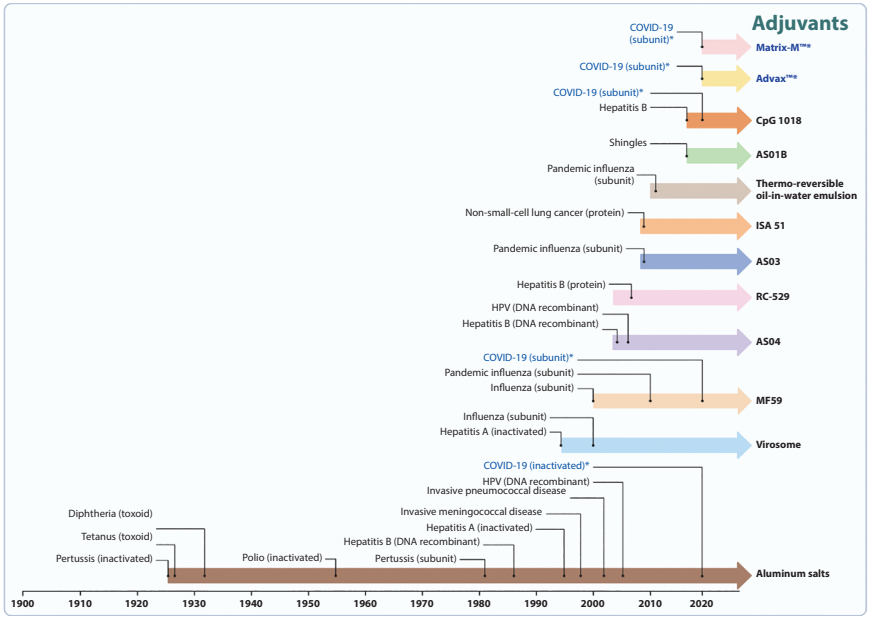

Figure 1.1 A timeline of vaccine adjuvant development over the last 100 years and used in humans. Figure adapted by content experts Iwasaki, A., Lee, J-H. and Omer, S.B at Biorender.com as Figure 1.1 in https://www.cell.com/cell/pdf/S0092-8674(20)31327-X.pdf

adhesive molecules, enhancing antigen presentation and have been used in malaria and influenza vaccine studies [10–12].

b. **QS-21:** QS-21 is a potent vaccine adjuvant sourced by extraction from the Chilean soapbark tree (*Quillaja saponaria*). QS-21 exhibits freeze-thaw stability and has shown promise as an adjuvant for inducing specific CD8+ T-cell responses and exhibits minimal toxicity [13]. Quil A is also derived from *Quillaja saponaria* tree.

c. **Monophosphoryl Lipid A:** A derivative of lipopolysaccharide has been used as an adjuvant in vaccines to enhance antibody and T-cell immune response to antigens. Monophosphoryl lipid A binds to Toll-like receptor 4 (TLR4) on antigen-presenting cells stimulating pro-inflammatory cytokines, maturation, and activation of antigen-presenting cells [14, 15]. AS04 is the best known formulation, which incorporates both monophosphoryl lipid A and aluminum hydroxide.

d. **Ribi Formulation:** Incorporating mycobacterial cell walls and monophosphoryl lipid A, this formulation has

demonstrated superior antibody titers and both humoral and cellular immune responses compared to aluminum hydroxide adjuvants [14, 16, 17].

e. **Squalene-Based Adjuvants:** MF59 by Novartis approved in 1997 and AS03 by GlaxoSmithKline approved in 2013 are oil-in-water emulsions containing squalene, a naturally occurring lipid. MF59 and AS03 adjuvants enhance antigen uptake by antigen-presenting cells and stimulate immune cells at the injection site, resulting in activation of both humoral and cell-mediated immune responses. MF59 is used in seasonal influenza vaccines for older adults, whereas AS03 is used in some pre-pandemic (H5N1) and pandemic influenza vaccines [5, 18–20].
f. **Bacterial Toxoids:** Toxoids, such as detoxified forms of tetanus and diphtheria toxins, can serve as adjuvants when co-administered with antigens [21]. They provide T cell help and can enhance the immune response to the co-administered antigen as were shown to be effective in anti-cancer peptide based vaccines [22–27].
g. **Mineral Salts:** Besides aluminum salts, other mineral salts like calcium phosphate and calcium carbonate have been used as adjuvants to stabilize antigens and enhance their immunogenicity. These salts can adsorb antigens and facilitate their uptake by antigen-presenting cells.

Conventional adjuvants have been instrumental in the success of several vaccines by improving their efficacy and durability. However, they often have limitations, such as inducing primarily humoral immune responses or having reactogenicity concerns, which have driven the search for novel adjuvants with improved safety profiles and broader immunostimulatory capabilities.

1.4 Particulate Adjuvants

Particulate adjuvants are a class of adjuvants that consist of particles designed to enhance the immune response to co-administered antigens. These adjuvants are often formulated as nanoparticles, liposomes, or other particulate structures to improve antigen delivery, uptake by antigen-presenting cells and subsequent activation of the immune system. Some notable types of particulate adjuvants include the following:

a. **Liposomes:** Liposomes, phospholipid-based vesicles, have been extensively studied since the 1970s for targeted drug delivery and immunoadjuvant applications [28]. They offer a versatile platform for adjuvant design, with the ability to entrap antigens, cytokines, and other immunomodulators [29]. As such, enhanced immune responses with liposome-entrapped antigens compared to free antigens have been shown, including some of the original studies 30 years ago against influenza virus A/PR/8 envelope proteins [30, 31]. Liposomal-based vaccines hold promise for more effective and tailored approaches in the design of vaccines against infectious diseases, cancer, and other health challenges.
b. **ISCOMs (Immunostimulatory Complexes):** ISCOMs, composed of Quil A adjuvant and peptides, achieve enhanced antigen immunogenicity with reduced adjuvant concentrations. These complexes induce both humoral and cell-mediated immune responses and have demonstrated promise in various animal models, including vaccines against hepatitis B, hepatitis C, influenza virus, malaria, human immunodeficiency, and certain veterinary vaccines [32–37].
c. **Emulsions:** Emulsion-based adjuvants, such as MF59 and AS03, consist of oil-in-water or water-in-oil formulations. They can stabilize antigens, promote their uptake by antigen-presenting cells, and enhance immune responses, particularly in elderly individuals. Indeed, MF59 is used in seasonal influenza vaccines for elderly individuals who have not responded to standard influenza vaccines [19, 38, 39]. In addition, MF59 has been evaluated in vaccines against meningococcus B, SARS-CoV-2, and malaria [40].
d. **Virosomes:** Virosomes are reconstituted viral envelopes devoid of viral genetic material. They can encapsulate antigens and fuse with cell membranes, facilitating antigen delivery and uptake by antigen-presenting cells. Virosomes are used in vaccines like Inflexal V for influenza [41].
e. **Nanoparticles:** Nanoparticles made of biodegradable polymers or inorganic materials can be used to encapsulate antigens and/or adjuvants [42]. These nanoparticles can protect antigens from degradation, target them to specific

cell types, and promote antigen uptake and presentation by antigen-presenting cells [43–45].

f. **Microparticles:** They are larger than nanoparticles but smaller than cells and can be made from various materials, including polymers and proteins. They can be designed to secrete antigens and adjuvants in a controlled manner, enhancing antigen presentation and immune stimulation. Key findings 30 years ago noted that size of the particle was important to stimulate different arms of the immune system [46].
g. **Nanogels:** Nanogels are hydrogel-based nanoparticles, which encapsulate antigens and adjuvants. They provide sustained release of encapsulated components, improve stability, and enhance antigen uptake and presentation by antigen-presenting cells.

Particulate adjuvants offer several advantages, including improved antigen stability, targeted delivery, and enhanced immune stimulation. They are being studied for use in various vaccines against infectious diseases, cancers, and other conditions to improve vaccine efficacy and facilitate the development of novel vaccine formulations.

1.5 Immunostimulatory Adjuvants

Immunostimulatory adjuvants enhance the immune response by directly activating immune cells or signaling pathways. Such examples include the following:

a. **TLR Agonists:** TLR agonists, such as Poly I:C (TLR3) and R848, mimic pathogen-associated molecular patterns to stimulate innate immune responses [47]. In addition, imiquimod, a synthetic imidazoquinolinone compound, binds to TLR7 stimulating pro-inflammatory immune responses. Alum Plus is an improved adjuvant, which combines aluminum salts with TLR agonists.
b. **Monophosphoryl Lipid:** Stimulates immune cells via binding to TLR4.
c. **Cytokines:** Incorporation of cytokines into adjuvants and vaccine formulations has been shown to enhance immune

responses, such as interleukin (IL)-1, IL-2, interferon (IFN)-gamma, and granulocyte-macrophage colony-stimulating factor (GM-CSF).

d. **CpG Oligodeoxynucleotides:** These synthetic DNA sequences stimulate TLR9, promoting a Th1-biased immune response. CpG adjuvants are being investigated for various vaccines, including those against infectious diseases and cancer [48–51].

1.6 Approved Adjuvants for Human Use

Improved adjuvants are essential for enhancing the efficacy of vaccines by boosting the immune response to antigens. While aluminum salts have been key in human vaccines, their limitations, such as variable efficacy and lack of cell-mediated immune stimulation, have directed research into novel adjuvant formulations (Figure 1.1). Several adjuvants have been approved in human vaccines:

a. **AS01:** The adjuvant used in the Shingrix vaccine against shingles is a combination of monophosphoryl lipid A and QS21 inducing robust immune responses even in older adults [52–54].

b. **AS03:** AS03 adjuvant is an oil-in-water emulsion based on squalene, a natural lipid, polysorbate 80, and a-tocopherol, a form of vitamin E. It was approved in 1997 by Novartis to be used in influenza vaccines. AS03 was also present in the Pandemrix influenza vaccine of the H1N1 influenza pandemic 2009-2010. Even though Pandemrix showed strong immune responses and protection against H1N1 infections, there were increased risks of narcolepsy. As such, the use of Pandemrix was discontinued in several countries, and alternative vaccines without the AS03 adjuvant were used for subsequent influenza seasonal and pandemic response vaccines.

c. **AS04:** This adjuvant combines aluminum hydroxide with monophosphoryl lipid A, a detoxified derivative of bacterial lipopolysaccharide, which has been shown to enhance both humoral and cell-mediated immune responses especially in human papilloma virus (HPV) vaccines. Indeed, Cervarix vaccine against HPV types 16 and 18; the Gardasil vaccine against HPV types 6, 11, 16, and 18; and Gardasil

9 against HPV types 11, 16, 18, 31, 33, 45, 52, and 58 comprise the adjuvant AS04 [55–58].

d. **MF59:** This is composed of squalene oil, polysorbate 80, and sorbitan trioleate and has been used in seasonal influenza vaccines. MF59 stimulates immune response, particularly in older individuals who have reduced response to standard influenza vaccines.

e. **Matrix-M:** A saponin-based adjuvant, purified from *Quillaja saponaria* Molina tree, combined with cholesterol and phospholipids to form 40-nm–like nanoparticles. Matrix-M enhances Th1 and cellular immune responses to several antigens and has a favorable safety profile. In fact, the Novavax (NVX-CoV2373) COVID-19 vaccine includes matrix-M adjuvant [59].

f. **Virosomes:** These are reconstituted viral envelopes containing no viral genetic material but capable of fusing with cell membranes and antigen uptake. Virosomal adjuvants are used in the influenza vaccine, Inflexal V, to enhance immune responses [41, 60–63].

1.7 Conclusion

The history of vaccination, spanning over a millennium, has witnessed remarkable advancements, from early inoculations in China to the modern era of sophisticated vaccine technologies. Edward Jenner and Louis Pasteur laid the foundation for vaccine development, whereas the identification of specific antigens enabled the creation of subunit and extract vaccines. Despite these strides, challenges such as safety concerns and limited immunogenicity persist, driving the need for innovative solutions like adjuvants. Adjuvants, crucial in modern vaccine development, enhance immune responses, making vaccines more effective and enabling the use of novel technologies. While conventional adjuvants like aluminum salts have been foundational, their limitations have spurred research into safer and more efficient options. Particulate adjuvants, such as liposomes and ISCOMs, offer improved antigen stability and targeted delivery, whereas immunostimulatory adjuvants like TLR agonists and cytokines directly activate immune cells, enhancing vaccine efficacy. Approved adjuvants, AS01, AS03, AS04, Matrix-M, MF59, and virosomes, have revolutionized vaccine formulations, enhancing immune responses against diseases like shingles, influenza, and HPV. Matrix-M™ in the Novavax COVID-19

vaccine exemplifies the potential of innovative adjuvants in pandemic responses. In conclusion, the ongoing evolution of vaccine technologies and adjuvants holds promise for safer and more effective vaccines. In the face of persistent global health challenges, enhancing vaccine design and delivery is essential to ensure the well-being of people worldwide.

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