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A COMPREHENSIVE REVIEW OF VACCINE ADJUVANTS: CURRENT APPLICATIONS, DEVELOPMENT, AND IN SILICO DESIGN

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Abstract

Adjuvants play a pivotal part in modern vaccine formulations by enhancing immune responses, prolonging protection, and reducing the required antigen dose. Although several adjuvants have been globally licensed, the development of novel adjuvants still faces major challenges such as unpredictable immunogenicity, potential toxicity, and high in vivo testing costs. In silico approaches offer promising solutions for accelerating adjuvant design and validation in a more efficient and targeted manner. This review highlights recent progress in computational strategies for adjuvant design, encompassing molecular docking, molecular dynamics simulation, epitope prediction, and the use of artificial intelligence. It also discusses currently licensed adjuvants and highlights case studies involving in silico-designed immune-receptor agonists such as Toll-like-receptor (TLR) ligands. Integrating empirical and bioinformatic strategies is expected to create new opportunities for developing safer, more specific, and personalized vaccine adjuvants. Key challenges and future research directions are also identified to optimize the incorporation of in silico approaches into global vaccine innovation.

Keywords: Adjuvant, In Silico, Vaccine

Abstrak

Adjuvan menjadi komponen penting dalam formulasi vaksin modern karena berfungsi untuk meningkatkan respons imun, memperpanjang durasi perlindungan, serta mengurangi dosis antigen yang dibutuhkan. Meskipun berbagai adjuvan telah disetujui penggunaannya secara global, pengembangan adjuvan baru masih menghadapi tantangan seperti ketidakpastian imunogenisitas, toksisitas, dan tingginya biaya uji in vivo. Pendekatan in silico menjadi solusi yang menjanjikan dalam mempercepat desain dan validasi adjuvan secara lebih efisien dan terarah. Artikel ini meninjau perkembangan terkini dalam pendekatan komputasi untuk perancangan adjuvan vaksin, meliputi *molecular docking*, simulasi dinamika molekul, prediksi epitop, dan pemanfaatan kecerdasan buatan. Selain itu, dibahas pula adjuvan yang telah berlisensi, serta studi kasus pemanfaatan pendekatan *in silico* dalam pengembangan agonis reseptor imun seperti TLR. Kombinasi antara pendekatan empiris dan bioinformatika diyakini akan membuka peluang baru dalam pengembangan adjuvan yang lebih spesifik, aman, dan dapat dipersonalisasi. Artikel ini juga mengidentifikasi tantangan dan peluang riset masa depan untuk mengoptimalkan integrasi pendekatan *in silico* dalam inovasi vaksinasi global.

Kata Kunci: Adjuvan, *In silico*, Vaksin

1. Introduction

Vaccination is one of the greatest achievements in medicine, having saved millions of lives from infectious diseases and effectively preventing dangerous infections such as measles, diphtheria, hepatitis, and influenza (Kayser & Ramzan, 2021). In 2021, the World Health Organization (WHO) confirmed that vaccines prevent approximately two to three million deaths each year (Toor et al., 2021). Vaccines contain antigenic substances composed of weakened or killed strains of viruses or bacteria. When administered into the human body, the general impact is asymptomatic, meaning

infection occurs, but it generates long-term immunity similar to that detected in individuals who have recovered from natural infections (Coffman et al., 2010).

The effectiveness of vaccines depends greatly on their ability to stimulate a strong, specific, and long-lasting immune response. In this regard, adjuvants, as non-antigenic substances co-administered with antigen, play a crucial role as immune enhancers to boost the immunogenicity of vaccines. Adjuvants allow for reduced antigen doses, diminish the need for booster immunizations, accelerate and prolong immune responses, and enhance vaccine efficacy in poor responders (Romerio et al., 2023). Despite their importance, the availability of adjuvants that are approved for use in human remains very limited due to stringent requirements for safety, proven efficacy, and regulatory approval.

Currently, only a few types of adjuvants are widely used in licensed vaccines. Alum represent the oldest and most extensively applied adjuvant, functioning primarily through depot formation and induction of local inflammation. MF59 and AS03, which contain squalene, strengthen immune responses by stimulating the recruitment and activation of antigen-presenting cells. Saponin-based adjuvants, exemplified by QS-21 as a component of AS01, stimulate strong humoral and cellular immunity. Furthermore, immune receptor agonists such as Toll-like receptor (TLR) ligands—most notably CpG 1018 targeting TLR9—act as pathogen-associated molecular pattern (PAMP) mimics to directly trigger innate immune pathways (Zhao et al., 2023). The efficacy of vaccines can be augmented by conventional adjuvants, yet challenges persist, such as heightened reactivity, local reactogenicity, restricted ability to elicit cellular immunity, and the demanding, time-intensive nature of their development and validation (Petrovsky, 2015; Wilson-Welder et al., 2009).

A new computational approach known as in silico has emerged alongside advancements in biotechnology and bioinformatics. This approach encompasses various methods such as epitope prediction, molecular docking (predicting the binding and free energy of the corresponding complex between ligands and targets), molecular dynamics simulations, and the integration of artificial intelligence (AI) and machine learning in the modeling and design of vaccines and their adjuvants. The in silico approach allows for rapid, cost-effective simulations of antigen-adjuvant interactions with immune receptors without involving animal testing in the early stages. Furthermore, this approach supports the selection of more selective and rational adjuvants based on chemical properties, structure, toxicity, and predictions of immunogenicity (Wu et al., 2025; Hashempour et al., 2024).

Nevertheless, there remain several research gaps, such as limitations in predicting long-term toxicity, variations in immune responses among individuals, and the lack of adjuvants specifically designed for specific groups, such as immunocompromised patients or the elderly (Ranzani et al., 2021). Additionally, the emergence of AI and deep learning opens new opportunities to address these limitations, but its implementation in vaccine adjuvant design is still limited and not extensively reviewed systematically (Olawade et al., 2024).

This paper aims to review the in silico approach in the design and validation of vaccine adjuvants, covering the techniques used, case studies of success, and opportunities for integrating AI technology in the future. This article also presents a critical analysis of the challenges still faced in the prediction and validation of computationally based adjuvants. It is hoped that through the in silico approach, when combined with epitope prediction techniques and AI integration, it can produce vaccine adjuvants that are more effective, specific, and efficient compared to conventional approaches.

2. Methodology

A qualitative literature-based approach was applied in writing this review article. Also, a comprehensive search of scientific databases (PubMed, Scopus, Web of Science, and Google Scholar) was performed to identify relevant peer-reviewed articles, review papers, and clinical reports on vaccine adjuvants and in silico design methods. Several keywords were used, such as "vaccine adjuvants", "alum", "MF59", "AS01", "in silico adjuvant design", "bioinformatics", and "immunoinformatics". The search was limited to articles published in English between 2000 and 2024, although some landmark studies published earlier were included for historical context. Inclusion criteria consisted of studies that discussed the mechanism, application, development, or computational modelling of adjuvants in human or preclinical vaccine research. Exclusion criteria

included non-peer-reviewed sources, editorials, and studies without sufficient scientific detail. Selected articles were analyzed for content related to: (1) currently available adjuvants, (2) development history and mechanistic insights, and (3) recent advances in computational methods for adjuvant design and validation (Figure 1). Emphasis was placed on the integration of in vitro/in vivo findings with in silico models where applicable.

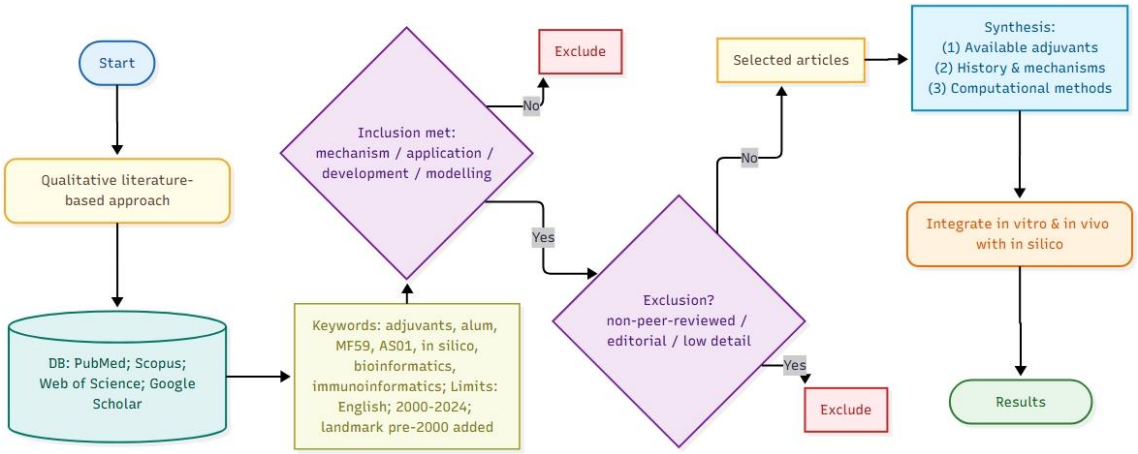

Figure 1. Research methodology flow diagram

3. Result and Discussions

An adjuvant is a component added to a vaccine to enhance the immune response to an antigen. The efficacy of a vaccine depends not only on its antigenic component but also on the adjuvant, which is more effective and often used to stimulate the immune system (Facciolà et al., 2022). Adjuvants do more than simply amplify the immune response. They have the ability to reduce the amount of antigen required in each vaccine dose, a phenomenon known as the dose-sparing effect (Molina Estupiñan et al., 2025). Due to increased immunogenicity, lower antigen doses are sufficient to achieve the desired level of protection. With adjuvants, limited antigen supplies can be used to produce more vaccine doses, thereby accelerating population vaccination coverage in public health emergencies, such as pandemics (Facciolà et al., 2022).

The mechanism of adjuvanted vaccines begins when the antigen and adjuvant in the vaccine are captured by Antigen-Presenting Cells (APCs). The adjuvant acts as a danger signal that activates APCs through various innate immune receptors, while also enhancing antigen presentation and cytokine release. Activated APCs then stimulate Helper T Cells, which coordinate two main branches of defense: driving B Cells to differentiate into Plasma Cells that produce antibodies (the humoral response) and supporting the activation of Cytotoxic T Cells through specific antigen presentation pathways (the cellular response). This process leads to the generation of Memory B Cells and Memory T Cells, enabling the body to mount a faster and stronger immune response upon re-exposure in the future. The illustration of the vaccine adjuvant mechanism can be seen in Figure 2.

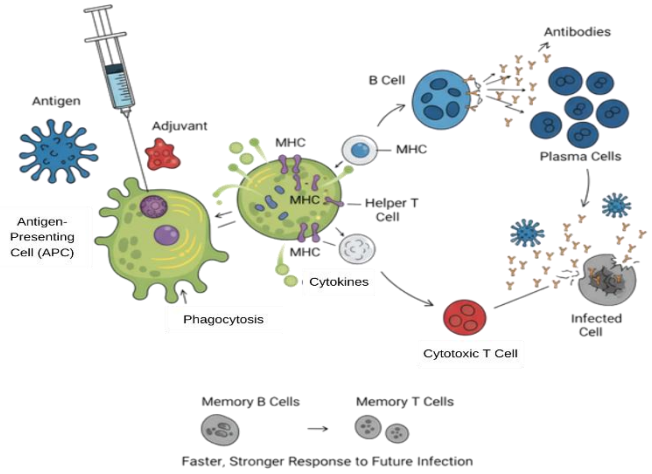

Figure 2. Ilustration of vaccine adjuvant mechanism

3.1 Current Available Adjuvant

3.1.1 Mineral Salts (Aluminum)

Among the various types of adjuvants, aluminum or alum is one of a mineral salt that has approved and licensed in the world (Ghimire, 2015). Alum has been approved in some human vaccines like hepatitis A and B, haemophilus influenza type b (Hib), human papilloma virus (HPV), diphtheria, tetanus, and meningococcal (Lee & Nguyen, 2015). These properties are gel-like precipitate of aluminum phosphate, aluminum hydroxide, unable to be solved (Cox & Coulter, 1997).

The mechanism of alum is precipitation by making the antigen released slowly because of the formation of an antigen depot at the inoculum site. So that the antibody response becomes strong (Kool et al., 2012). Alum is able to facilitate humoral immunity by Th2 cells in human (Didierlaurent et al., 2009). Besides the advantages of its safety, used and effective well well-tolerated, alum is also weak and rarely induces a cellular immune response (Brewer et al., 1996).

Transforming conventional aluminum (alum) adjuvants into nanoaluminum-based systems or refining their composition can significantly improve their efficacy (Zhao et al., 2023). Conventional alum has a micrometer size (1-10 μm) and a lower surface-to-volume ratio, making them less efficient at adsorbing antigens and more likely to induce a humoral response. On the other hand, nanoaluminum, with its nanometer size (200-600 nm) and higher surface-to-volume ratio, allows for much greater antigen adsorption and is more effective in triggering cellular responses. Furthermore, nanoaluminum is easier to sterilize and has broader potential applications in vaccine development, especially for stronger immune responses, although its safety and biodistribution still require further investigation (Lu & Liu, 2022). For these reasons, many researchers try to find a novel adjuvant by many approaches.

3.1.2 Oil in Water Emulsion (MF59, AS03)

Widely licensed in Europe for seasonal influenza vaccination (Vesikari et al., 2011), MF59 is also under clinical investigation for use with Human immunodeficiency virus (HIV), Herpes simplex virus (HSV), Cytomegalovirus (CMV) and HBV vaccines (Lee & Nguyen, 2015). It is an oil-in-water emulsion composed of squalene droplets stabilized with Tween 80 and Span 85, averaging 160 nm in diameter (Calabro et al., 2013). The adjuvant is recognized as safe, practical, and simple to formulate, with the added benefits of filtration sterilization and broad antigen compatibility. Its primary mechanism involves stimulating local immune activity, regulating chemokines and cytokines, recruiting CD11b+ and MHC II+ cells, and promoting antigen uptake by dendritic cells. This process

increases the overall number of antigen-presenting cells (APCs) at the injection site (Sivakumar et al., 2011). MF59 is better than alum adjuvant for influenza vaccine because it permits fewer doses and antigen dose sparing, induces stronger antibody responses, and creates marked memory responses, with both of Th1-Th2 cells. Surprisingly, MF59 has some side effects such as generates inflammatory arthritis and reactogenicity.

Another type of oil-in-water emulsion is AS03. It also includes as licensed vaccine adjuvant which contain squalene and alfa-tocopherol. AS03 improves not only humoral but also cell-mediated immunity. From 2009, this adjuvant is used in influenza vaccine for H1N1 and H5N1 pandemic (Gillard et al., 2014). AS03 has recently demonstrated strong clinical benefits in the development of the COVID-19 vaccine (Hager et al., 2022). While both emulsions demonstrate strong potential for adjuvant applications, more detailed analysis and optimization of their formulation components are still required.

3.1.3 TLR Agonist Molecule-based Adjuvant (AS04, CPG ODN 1018)

Monophosphoryl Lipid A (MPL), derived from the lipopolysaccharide (LPS) of *Salmonella minnesota* R595, is a potent adjuvant that is safe, well tolerated, and capable of enhancing immune responses to co-administered antigens (Ulrich & Myers, 1995). Acting as a Toll-like receptor 4 (TLR4) agonist, MPL serves as an immunostimulatory adjuvant. The AS04 formulation combines MPL with aluminum salts (Sivakumar et al., 2011), promoting pro-inflammatory cytokine production, including IL-2 and IFNγ, optimizing APC activation, and eliciting Th1 immune responses that alum alone cannot achieve (Lee & Nguyen, 2015). HPV and HBV vaccine are kinds of licensed AS04-adjuvanted vaccines for human (Garon et al., 2011; Fabrizi et al., 2015).

The synthetic single-stranded DNA CpG ODN 1018 has been extensively studied as a Toll-like receptor (TLR) agonist. By specifically activating TLR9, it initiates TRF7 signaling, leading to the production of type I interferons and pro-inflammatory cytokines, which in turn drive strong Th1 responses and the induction of cytotoxic T cells. Because of this, it can elicit a stronger cellular immunological response than adjuvants made of aluminum. Initially, CpG ODN 1018 was authorized for use in HBV vaccinations. As a possible vaccination adjuvant for the COVID-19 vaccines, CpG ODN 1018 is presently undergoing clinical trials (NCT04450004, NCT04405908). SCB-2019, a CpG ODN 1018-adjuvanted COVID-19 vaccine, has recently been assessed for use in emergency situations (Zhao et al., 2023).

3.1.4. AS01

The adjuvant AS01 is currently licensed for vaccines against herpes zoster and respiratory syncytial virus in elderly populations, as well as for malaria vaccines in children (Zhao et al., 2023; Khosasih, 2023). Structurally, AS01 is a liposome-based system incorporating two immunostimulants: MPLA and QS-21, a saponin extracted from *Quillaja saponaria*. It provides a dual role—facilitating antigen presentation and enhancing immune stimulation (Didierlaurent et al., 2009). The liposome component prevents antigen degradation, improves antigen bioavailability, and supports stronger recognition by antigen-presenting cells (APCs). MPLA and QS-21 as immunostimulatory function of AS01 are formulated together. Through TLR4, MPLA activates the innate immune system, increasing Th1-type responses. In addition, QS-21 stimulates caspase 1 in subcapsular sinus macrophages (SSMs) and NLRP3 in APCS (Pulendran et al., 2021). It stimulates caspase 1 to increase the synthesis of active versions of the cytokines IL1β, IL18, and IL33. Furthermore, QS-21 has been shown to facilitate cross-presentation and endosomal escape (Zhao et al., 2023). Recently, AS01 was used to create a new peptide vaccine against tuberculosis (Tait et al., 2019). Although much has been learned in preclinical models, there is still much to learn about how AS01 functions in humans, particularly in older subjects.

Table 1. Summary of Current Available and Lisenced Adjuvant Vaccines

Adjuvant	Composition/Category	Mechanism of Action	Used in Vaccines	Regulatory Status
Aluminum	Aluminum hydroxide or Aluminum phosphate	Activation of inflammasome NLRP3, depot effect	Hepatitis B (HBV), DPT, HPV	Globally approved (FDA, EMA); GlaxoSmithKline, Sanofi
MF59	Squalene, Tween 80, and Span 85	Activation of APC, increased antigen uptake	Influenza (Fluad)	Approved (EMA, FDA); Novartis → Seqirus
AS03	Squalene emulsion, α-tocopherol, and tween 80	Cytokine induction and DC activation	Pandemrix (H1N1), COVID-19 (clinical trials)	Approved (EMA); GlaxoSmithKline
AS04	Alum and MPL (TLR4 agonist)	Activation of TLR4, Th1 response induction	Cervarix (HPV), Fendrix (HBV)	Approved (EMA, FDA); GlaxoSmithKline
CPG ODN 18	Cytosine phosphoguanine (CpG), a synthetic form of DNA that mimics bacterial and viral genetic material	Activation of TLR9, IFN-α, Th1	HEPLISAV-B (Hepatitis B)	Approved (FDA); Dynavax Technologies
AS01	Monophosphoryl lipid A (MPL) and QS-21 extracted from the bark of Quillaja Saponaria (QS), combined in a Liposomal formulation	APC activation, enhanced immune memory	Shingrix (Herpes Zoster), Malaria	Approved (EMA, FDA); GSK (AS01 combination)

3.2 Development and General Mechanisms of Adjuvants

Adjuvants are components or substances added to vaccines to enhance the immune response to antigens. The addition of adjuvants strengthens the ability of antigens to stimulate the formation of antibodies (Pulendran et al., 2021). For over a century, adjuvants have been used as key components of many inactivated and subunit vaccines.

The first adjuvant was identified in 1925 by Gaston Ramon, who utilized a mixture of various substances, including starch (tapioca), to enhance the immune response to the diphtheria vaccine (Chippaux, 2024). In 1926, Alexander Glenny demonstrated that combining aluminum salts with antigens produced significantly higher antibody levels compared to antigen administration alone (Zhao et al., 2023). Although Freund developed a water-in-oil emulsion (Freund's adjuvant) in 1940, its use, like that of bacterial lipopolysaccharides introduced in 1956, was not approved in human vaccines due to toxicity concerns. Consequently, aluminum adjuvants remained the only adjuvants approved for human vaccines between the 1920s and the 1990s.

The development of adjuvants accelerated after the licensing of MF59 for influenza vaccines in 1997. Four other adjuvants have been approved for human vaccines in the last 20 years, including AS04 in 2005 for the hepatitis B and the human papillomavirus vaccine, AS03 in 2009 for the influenza vaccines, AS01 in 2017 for the herpes zoster vaccine and the malaria vaccine, and Cytosine phosphoguanosine (CpG) 1018 in the same year for the hepatitis B vaccine Heplisav-B (Pulendran et al., 2021). Recently, CpG 1018 was combined with alum in the COVID-19 vaccine IndoVac Indonesia (Maddeppungeng et al., 2024) and saponin nanoparticles Matrix-M that enabled the COVID-19 vaccine Novavax NVX-CoV2373 (Stertman et al., 2023). The timeline of vaccine adjuvant development can be seen in Figure 3.

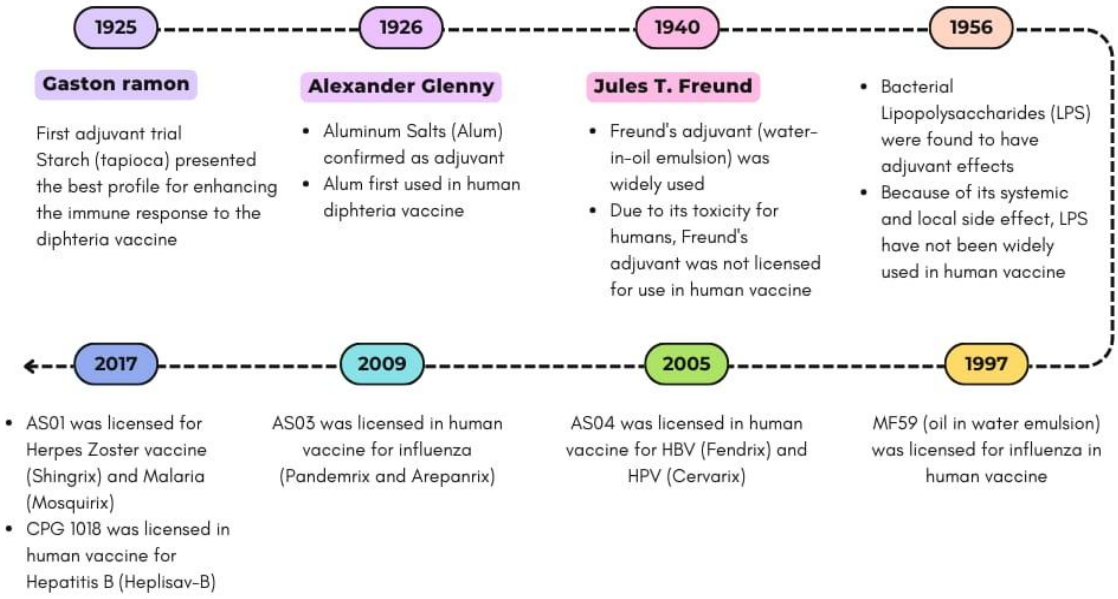

Figure 3. Timeline of Vaccine Adjuvant Development

According to Zhao et al. (2023), adjuvants have two main mechanisms of action: immunostimulation and delivery systems. In immunostimulation, adjuvants act as danger signal molecules that activate antigen-presenting cells (APCs) by targeting pattern recognition receptors (PRRs), such as Toll-like receptors (TLRs). This process generates two main signals: signal 1, where antigen presentation occurs through major histocompatibility complex (MHC) presented on the surface of APCs after the antigen is taken up and processed; and signal 2, which includes co-stimulatory molecules (such as CD40, CD80, CD86) and inflammatory cytokines (such as IL-6, IL-12).

Adjuvants in delivery systems enhance vaccine efficacy by prolonging antigen release, forming depots at injection sites, and improving uptake by antigen-presenting cells through particle size and structural modifications, thereby boosting antibody production and T cell activation (Zhao et al., 2023). Recent advances focus on nano-formulations, where polymer nanoparticles (e.g., PLGA, POx) and self-adjuvanted nanovaccines enable controlled release, lymph node targeting, and co-delivery of immunostimulants, while virosomes and virus-like particles provide intrinsic immune activation signals, offering safer, more effective vaccines and promising improvements in global health (Xing et al., 2025; Moni et al., 2023).

A key challenge in developing new adjuvants is ensuring they progress beyond preclinical studies, as safety remains the primary limitation for human use. Over decades, research has reduced adjuvant toxicity while emphasizing properties like biodegradability, biocompatibility, and non-immunogenicity (Pasquale et al., 2015; Guy, 2007). However, issues such as limited adjuvanticity, animal model reliability, and antibody testing persist, highlighting the need for innovative strategies like in silico design to overcome these barriers (A. Gupta & Chaphalkar, 2015).

3.3 In Silico Approach in the Design and Validation of Adjuvants

The development of bioinformatics and molecular structure computation has opened new pathways in the discovery and development of adjuvants through in silico approaches. This strategy allows high-throughput screening of molecular candidates much faster than conventional experimentation. In this context, databases act as repositories of validated vaccine-related data, while computational tools provide predictive frameworks for designing novel adjuvants and vaccine components (He & Xiang, 2013).

Toll-like receptors (TLRs), Stimulator of Interferon Genes (STING), and NOD-like receptors (NLRs) are Pattern Recognition Receptors (PRRs) that play essential roles in the early activation of the innate immune system. Specific agonists that activate PRRs will result in cytokine production, maturation of dendritic cells, and activation of adaptive T cells, which will guide and improve the immune response to vaccine antigens (Putri & Putri, 2023). Therefore, PRR agonists have become one of the main strategies in modern adjuvant design.

Epitope-based approaches have gained attention in vaccine development due to their ability to elicit specific, targeted, and safe immune responses. Epitopes, whether from B or T cells, are fragments of antigens recognized by the immune system. The precise selection of epitopes not only determines the effectiveness of the vaccine but also influences the need for adjuvants to enhance and balance the types of immune responses generated.

Bioinformatics is widely used to identify candidate vaccines for the Zika virus (ZKV) from conserved polyvalent B-cell epitopes on the viral glycoprotein (Kharisma et al., 2020), design epitope-based vaccines against meningitis-causing bacteria (Zahroh et al., 2016), design vaccines for H5N1 based on predictions of B and T cell epitopes (Tambunan et al., 2016), and analyze the dengue virus envelope (Tambunan et al., 2009).

A study conducted by Firmansyah et al. (2021) utilized an in silico approach to design an epitope-based vaccine targeting the Spike glycoprotein of SARS-CoV-2. The researchers performed simulations of vaccine-adjuvant interactions with TLR3 and TLR4 using molecular docking (HDOCK and PyDock). This receptor-based structural approach is relevant to adjuvant development. Antigenicity predictions of protein sequences were performed using the Vaxijen 2.0 web server, while epitope predictions and molecular binding analyses between the epitope and alleles were conducted using the NetCTL 1.2, Tepitool IEDB, and HDOCK web servers. Immunogenicity evaluations using C-IMMSIM were performed for in silico validation of adjuvant immune effectiveness (Firmansyah et al., 2021).

Additionally, Ali et al. (2022) designed a peptide vaccine against non-typhoidal Salmonella by incorporating Csga, a structural protein from Salmonella fimbriae (TLR2 agonist), as an adjuvant component in the vaccine construct. This strategy can also be applied to the development of peptide-based or fusion domain adjuvants. The vaccine-TLR2 complex was modeled using Hawkdock and validated through molecular dynamics simulations (MD) using YASARA with the AMBER14 force field. The immunostimulatory effects were validated with C-ImmSim to predict humoral and cellular immune responses. This simulation can be used to compare the immunogenic performance of various adjuvants before biological testing (Ali et al., 2022).

Several TLR5 agonists as new vaccine adjuvants can also be determined by designing various derivatives of flagellin. The 3D structure for flagellin derivatives was created using the I-TASSER online server. Gromacs96 implemented in Swiss-PDB Viewer v.4.2 was used to correct distorted geometries by minimizing energy. Additionally, using Chimera 1.10.1 software, the loops from the minimized energy constructs were filtered (Pettersen et al., 2004). Docking analyses to predict fusion protein interactions were determined using Hex software (Farhadi et al., 2016).

Research conducted by Giulini et al. (2024) found that HADDOCK could produce accurate models of antibody-antigen complexes using antibody structures generated by antigen structures predicted by AlphaFold2. Targeted docking using knowledge about complementary determinant regions on antibodies and some information about the targeted epitopes allowed for the creation of high-quality complex models with reduced sampling, resulting in a computationally inexpensive protocol that outperformed the ZDOCK baseline (Giulini et al., 2024). Furthermore, Rani (2024) developed a multi-subunit epitope vaccine against Cyprinid herpesvirus using GROMACS for Molecular Dynamics (MD) simulations (Rani et al., 2024).

According to Nagpal (2017), they predicted antigen-presenting cell modulators for designing peptide-based vaccine adjuvants using bioinformatics (Nagpal et al., 2017). The prediction model was built using Support Vector Machine (SVM), which can develop a model for predicting epitopes, especially T cell epitopes (Huang, 2005). This supports an effective model at high dimensionality. To validate the results, they divided the dataset into two types, internal and external.
Based on internal validation, the dataset was divided into five sets, four sets were used to train the model, and one set was used to test the model. This was repeated five times. For external validation, the best model from the five previous cross-validations was tested. Standard metrics (Sensitivity, Specificity, Accuracy, and Matthews Correlation Coefficient) were used to measure the model's performance (S. Gupta et al., 2013).

Chimeric peptides HA2/Mx were developed for creating adjuvanted vaccines through in silico analysis. HA2/Mx chimera was designed by fusing with each Mx motif using hydrophobic amino acid linker repeats (EAAAK). Subsequently, a Kozak sequence was introduced to enhance translation initiation efficiency. The constructed HA2/Mx was identified using Prot-Param (Soleimani et al., 2015). Model validation was built using ProSA, which provides overall model quality based on C-alpha positions.

3.4 Case Studies of Success in In Silico-Based Adjuvants

The development of in silico approaches in the design of vaccine adjuvants has shown significant progress, ranging from simple prediction techniques to complex multidimensional computational strategies based on artificial intelligence (AI).

Romerio et al. (2022) used conventional molecular docking approaches with AutoDock Vina to screen glucosamine-based adjuvant candidates as TLR4 agonists. Through interaction simulations in three different binding modes, followed by molecular dynamics (MD) for 200 ns, this study successfully identified two candidate molecules (FP20 and FP22) that were stable in the TLR4-MD2 complex. In vitro and in vivo results showed that both candidates were able to induce a strong immune response, particularly the production of TNF-α and IL-1β in human PBMCs, along with a significant increase in IgG titers in mice. This approach illustrates a classic strategy in molecular design that is effective and efficient, although it has limitations in atomic interaction resolution and in understanding the dynamics of larger biological systems (Romerio et al., 2023).

Strobl et al. (2022) showcased a modern structure-guided approach by using crystallographic data (PDB 3FXI) to design a TLR4 agonist from an unnatural disaccharide, integrating molecular dynamics simulations and stereochemical considerations to enhance affinity and efficacy, while Martin et al. (2024) advanced in silico methods further by combining AlphaFold-based enzyme mining, flux balance analysis, and metabolic pathway design to reconstruct the QS-21 biosynthetic pathway in tobacco, enabling sustainable large-scale production and genomic-level optimization, together highlighting a shift from static docking to systemic, AI-driven adjuvant design in vaccine development (Martin et al., 2024)

3.5 Challenges and Limitations of The In Silico Approach

Although the in silico approach shows good results in designing and discovering new vaccine adjuvant candidates, validation through in vitro and in vivo studies is still required before human use. Studies on HPV indicate that in silico results must be validated in the laboratory (Tambunan et al., 2010). This is because there are still challenges in predicting the toxicity, allergenicity, and pharmacokinetics of adjuvants. Methodologies such as AllergenFP and ToxinPred that are used remain limited in their accuracy (Guo et al., 2025). Additionally, the computational scale and accessibility of advanced software remain challenges for researchers conducting in silico studies. Most simulations (dock/MD) use public servers such as ClusPro and iMODS, but access and speed continue to be obstacles.

3.6 Future Direction and Recommendations

Advancements in computing and artificial intelligence (AI) are reshaping vaccine development, particularly in adjuvant design and validation, with in silico approaches evolving into comprehensive big data systems capable of supporting real-time, high-precision, and personalized vaccine design. By combining computational simulations with machine learning (ML) methods such as Bayesian and black-box optimization, researchers can accelerate adjuvant screening, improve accuracy, reduce reliance on biological testing, and lower development costs by up to tenfold, significantly enhancing the speed and efficiency of vaccine innovation (Kim et al., 2023).

Based on a narrative review written by Olawade et al. (2024), the use of AI in machine learning and deep learning plays a crucial role in accelerating antigen design, epitope prediction, and identification of new adjuvant candidates with high computational efficiency.

AI accelerates adjuvant discovery by analyzing molecular interactions and immune profiles to identify promising molecules from extensive libraries. Tools including SAR modeling, docking simulations, and virtual screening accelerate the refinement of adjuvant formulations, improving their safety, stability, and immune potency, as well as adapting them to particular antigens or population needs. Through its predictive capabilities, AI drives faster vaccine development for a range of conditions—infectious diseases, cancers, and autoimmune disorders—marking a significant shift in public health innovation (Olawade et al., 2024).

Tools like AdjuPred, which uses machine learning on large datasets of antigen-adjuvant interactions, forecast compatibility and effectiveness for vaccine design. Alongside the in-silico Adjuvant Discovery Platform, these systems streamline the search for candidates most likely to enhance immune responses, stability, and efficacy. Such AI-driven platforms allow researchers to prioritize optimal adjuvants for different formulations, significantly advancing rational vaccine development (Gude et al., 2025).

4. Conclusion

Adjuvants are essential for modern vaccines, particularly subunit and recombinant types that are less immunogenic, as they enhance both the effectiveness and duration of protection. Established adjuvants such as alum, MF59, AS03, AS04, AS01, and CpG ODN 1018 have proven critical in shaping adaptive immune responses and extending vaccine coverage to vulnerable groups like the elderly and immunocompromised. However, developing adjuvants through empirical methods is often time- and resource-intensive, with challenges in predicting toxicity and efficacy. To address this, combining empirical research with computational approaches such as epitope prediction, molecular docking, molecular dynamics simulations, and artificial intelligence offers a powerful strategy to accelerate discovery, optimize candidates, and enable more rational design grounded in molecular mechanisms.

Moving forward, the utilization of artificial intelligence, big data, and multi-omics integration opens up significant opportunities for generating more specific, safe, and tailored adjuvants according to individual immune characteristics. Furthermore, further research is needed to address predictive limitations regarding toxicity, allergenicity, and long-term clinical performance. With the support of increasingly advanced technologies and cross-disciplinary collaboration, in silico-based adjuvant development has the potential to transform the global vaccination landscape with greater precision and personalization.

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