Classification and Mechanism of Action of Immune Adjuvants: Postprint

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Abstract

Novel vaccines such as DNA vaccines, recombinant vaccines, and subunit vaccines, manufactured through modern biotechnology, although demonstrating enhanced safety profiles compared to conventional vaccines, exhibit inferior immunogenicity and require adjuvants to augment vaccine immunogenicity. As research on adjuvants has progressively advanced, various adjuvants including aluminum adjuvants, oil emulsion adjuvants, microbial adjuvants, propolis adjuvants, levamisole adjuvants, liposome adjuvants, traditional Chinese medicine adjuvants, and small peptide adjuvants have been successively developed, with their mechanisms of action becoming increasingly elucidated through continued investigation. Findings from animal immunization experiments reveal that small peptide immunological adjuvants not only augment specific immune responses, thereby functioning as immunopotentiators, but also offer advantages of facile acquisition, convenient transportation and storage, and high safety profiles, suggesting they may constitute a primary direction for future adjuvant research.

Full Text

Classification and Mechanism of Immune Adjuvants

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Abstract

Novel vaccines such as DNA vaccines, recombinant vaccines, and subunit vaccines developed through modern biotechnology, while offering improved safety profiles compared to conventional vaccines, often exhibit lower immunogenicity and require adjuvants to enhance their efficacy. As adjuvant research has progressed, various types have been developed, including aluminum adjuvants, oil

emulsion adjuvants, microbial adjuvants, propolis adjuvants, levamisole adjuvants, liposome adjuvants, traditional Chinese medicine adjuvants, and peptide adjuvants, with their mechanisms of action becoming increasingly clear. Animal immunization experiments have demonstrated that peptide immunoadjuvants not only enhance specific immune responses but also function as immune potentiators. Moreover, they are easy to obtain, transport, and store, and exhibit high safety profiles, making them a promising direction for future adjuvant research.

Keywords: Vaccine; immune adjuvant; mechanism of action

Introduction

Vaccination represents the most cost-effective approach for preventing and treating infectious diseases, with vaccine immunogenicity and safety being paramount concerns in this field. While conventional vaccines—prepared through artificial attenuation, detoxification, or inactivation—typically exhibit higher immunogenicity, they suffer from instability and carry potential risks of reversion to virulence. In contrast, novel vaccines such as DNA vaccines, recombinant vaccines, and subunit vaccines offer superior safety profiles. However, these vaccines have notable limitations, chief among them being their generally low immunogenicity, which fails to provide adequate immune protection. Consequently, adjuvants or delivery carriers are necessary to enhance their effectiveness, making the development of efficient, safe, and effective adjuvants to boost specific immune responses critically important [1].

Immunologic adjuvants are substances that non-specifically enhance or modify specific immune responses to matched antigens. They can increase antigen immunogenicity or alter the type of immune response, yet possess no antigenicity themselves [2], functioning as non-specific immune enhancers. Adjuvants can be broadly combined with various vaccines and offer numerous advantages, including reduced antigen dosage, rapid immune system activation, enhanced immune responses, and prolonged antigen release [3,4,5], thereby playing a vital role in improving vaccine efficacy.

1. Classification of Vaccine Adjuvants

As research on immune adjuvants has deepened, novel adjuvants continue to be discovered. Common immune adjuvants can be broadly categorized as follows:

1.1 Aluminum Adjuvants

In 1926, the adjuvant activity of aluminum compounds was confirmed, and they have been widely used for over ninety years. Aluminum adjuvants are milky-white gelatinous semisolids, with common forms including aluminum hydroxide gel, aluminum phosphate, aluminum sulfate, ammonium alum, and potassium

alum, though Al(OH) is most commonly used [6]. Al(OH) has extensive applications and remains the only adjuvant approved by the IDA for use in both humans and animals [7]. This adjuvant adsorbs protein antigens from solution, forming antigen precipitates that slowly release antigen after injection, thereby substantially prolonging antigen exposure time [8,9]. Additionally, it promotes macrophage responses at the injection site [10,11]. However, aluminum adjuvants have limitations, including weaker immune responses compared to other adjuvants and inability to elicit cell-mediated immunity [12]. Studies have revealed that aluminum adjuvants can enter the brain in rats, posing potential risks. They also exhibit biased immune response patterns (Th2-type) and significant local reactions.

1.2 Oil Emulsion Adjuvants

Oil emulsion adjuvants were successfully developed in 1936 with the creation of Freund' s complete adjuvant. The primary component of these adjuvants is oil, which classifies them into mineral oil and non-mineral oil adjuvants. Oil emulsion adjuvants promote high-titer antibody production against various antigens, extend continuous antigen stimulation, reduce required antigen dosage, and decrease vaccination frequency [13], leading to their widespread use in animal vaccines. However, these adjuvants carry safety concerns, including tissue damage, stress responses, mineral oil residues [14], and potential carcinogenicity [15]. Their mechanisms of action involve: (1) encapsulating specific antigens in oil for slow release while protecting them from enzymatic degradation, thereby generating sustained, high-level specific immune responses; and (2) stimulating local inflammatory reactions that promote immune cell proliferation and enhance immune responsiveness [16].

Common commercial oil emulsion adjuvants include Freund' s adjuvant, white oil Span adjuvant, MF-59 [17], ISA 206, ISA 720 [18], Adjuvant-65, and SAF. Water-in-oil adjuvants are predominantly used in veterinary vaccines, while oil-in-water adjuvants are common in human vaccines. All oil adjuvants require emulsification before use with vaccines. Common emulsification equipment includes colloid mills, high-pressure homogenizers, in-tank shear mixers, and emulsification tanks, with different adjuvants requiring specific processes. For ISA 206, the adjuvant-to-antigen mass ratio is 1:1. During addition, oil and aqueous phases are adjusted to appropriate temperatures, with antigen added at 1.6 million mL/h while mixing at 80 rpm. Homogenization pressure is set to appropriate low and high pressures, typically reaching 29°C after homogenization and cooling to 15°C before packaging.

1.3 Microbial-derived Adjuvants

Microbial components have long been reported to enhance immune responses. In the 1950s, lipopolysaccharides from Gram-negative bacteria were confirmed to possess adjuvant activity. As research progressed, numerous microorganisms demonstrated adjuvant efficacy, including *Mycobacterium*, certain *Bordetella*

pertussis, *Pseudomonas aeruginosa*, *Brucella*, *Escherichia coli*, and *Clostridium welchii* lipopolysaccharides, tuberculin, and Gram-positive bacteria such as *Staphylococcus*, *Corynebacterium parvum*, *Streptococcus*, and *Lactobacillus* [19]. Experiments show that these microorganisms or their products significantly enhance specific immune responses when co-administered with vaccines [20]. However, bacterial components are generally toxic, representing a major obstacle to their use as human vaccine adjuvants.

1.4 Propolis Adjuvants

In the 1960s, Soviet scientists demonstrated the adjuvant activity of propolis. Propolis is a fragrant, resinous solid material produced by bees from plant buds or trunk resins through a series of processing steps. Its composition is extremely complex, containing flavonoids, various amino acids, aromatic volatile oils, vitamins [21,22], and multiple trace elements. Propolis itself does not enhance immune responses but functions as an adjuvant when used with vaccines [23], enhancing immune responses as a natural immune potentiator with additional antibacterial properties [24]. Compared to traditional adjuvants like oil emulsions, propolis adjuvants offer superior safety [25].

1.5 Levamisole Adjuvants

In 1971, Renoux et al. discovered that levamisole combined with vaccines enhances immune efficacy, initiating research into its adjuvant activity. Levamisole is an imidazole derivative obtained through cyclization of racemic tetramisole and d-camphor-10-sulfonic acid followed by hydrolysis and salt formation. It is widely used in veterinary medicine as an anthelmintic against roundworms and hookworms. Studies show levamisole has adjuvant effects, improving immune response efficacy when combined with vaccines by inducing T cell differentiation into sensitized T cells that produce lymphokines such as MHF and MIF, ultimately activating macrophages. Levamisole can restore function in damaged peripheral immune cells, T cells, macrophages, and neutrophils. While generally having minimal side effects, long-term use can cause diarrhea and liver damage.

1.6 Liposome Adjuvants

In 1974, Allison et al. first reported the immune-enhancing effects of liposomes [26]. Liposomes are prepared from phosphatidylcholine and ceramide, featuring a bilayer structure. Research indicates that longer, more saturated phospholipid acyl chains produce more pronounced immune-enhancing effects [27]. As adjuvants, liposomes also function as carriers, resembling cell membrane microspheres [28]. Liposomal adjuvants offer numerous advantages: (1) high safety due to their composition from cell membrane-like materials, making them completely biodegradable without residues; (2) phospholipid structures containing both hydrophilic and lipophilic groups, enabling encapsulation of both hydrophilic and lipophilic antigens for broad applicability; (3) protection

of encapsulated antigens from degradation, allowing slow, continuous release for sustained immune stimulation; and (4) freezability for storage. However, disadvantages include mild side effects despite high safety, and inconsistent encapsulation efficiency for some antigens, potentially compromising vaccine efficacy.

1.7 Traditional Chinese Medicine Adjuvants

In 1984, QuilA was reported to have immune-enhancing effects. QuilA is a saponin extracted from the bark of the South American soap tree, but it has significant side effects and strong toxicity, causing granulomas and hemolysis. As research advanced, increasing numbers of traditional Chinese medicine components have been applied as vaccine adjuvants, including saponins, polysaccharides [29], and herbal formulas. (1) Saponins are steroid or triterpenoid glycosides widely found in terrestrial higher plants and some marine animals and bacteria. Many saponin components serve as adjuvants with antibacterial properties and high safety [30]. (2) Polysaccharide components from traditional Chinese medicine also function as effective adjuvants. Polysaccharides are sugar chains formed by at least ten monosaccharides linked by glycosidic bonds, with common sources including *Gastrodia*, licorice, *Astragalus* [32], *Cordyceps*, and *Ganoderma* [33]. These polysaccharides have minimal side effects, no cytotoxicity [34], and do not inhibit bone marrow hematopoietic cell proliferation or reduce anti-infection capacity [35,36]. (3) Herbal formulas also show adjuvant potential, with experiments demonstrating that formulas such as Yupingfeng Powder, Sijunzi Decoction, Jinji Powder, and Huanglian Jiedu Decoction significantly increase antibody titers in animal serum when combined with vaccines, indicating their potential to enhance immune efficacy and development prospects as vaccine adjuvants.

1.8 Peptide Adjuvants

Peptide adjuvants are a recently discovered class of substances that enhance immune efficacy. These are <10 kD peptides secreted by bacteria into supernatants and obtained through filtration. Animal immunization experiments have shown that these peptides significantly increase antibody titers, demonstrating clear adjuvant effects. Peptide adjuvants offer numerous advantages: easy acquisition, convenient storage and transport, and high safety due to their origin from probiotic secretion, making them promising candidates for human vaccine adjuvants.

2. Mechanism of Action

These adjuvants can be categorized by their mechanisms of action as follows:

2.1 Antigen Depot Effect

After local antigen injection, adjuvants recruit and store excess antigen, releasing it slowly and continuously [37]. This approach prolongs antigen stimulation, thereby increasing antibody titers [38] and improving immune response efficacy, as seen with liposome adjuvants.

2.2 Upregulation of Cytokine and Chemokine Expression

Cytokines are small proteins synthesized and secreted by cells upon stimulation, functioning as intercellular signaling molecules that regulate immune responses at very low concentrations. Chemokines are low-molecular-weight proteins (mostly 8-10 kD, such as IL-8 and MCP-1) that recruit leukocytes to infection sites [40,41]. In immune responses, cytokines and chemokines play crucial roles, and upregulating their expression enhances immune effects, as observed with microbial adjuvants and MF-59.

2.3 Increasing Antigen Surface Area

Some antigens have small volumes and low molecular weights insufficient to induce immune responses. Adjuvants encapsulate these antigens, increasing their molecular weight and surface area, thereby enhancing immunogenicity, as seen with oil emulsion adjuvants.

2.4 Carrier Function

Some adjuvants serve as carriers, encapsulating antigens to prevent enzymatic degradation and enabling continuous antigen release to increase antibody titers, as seen with aluminum adjuvants.

2.5 Promotion of Dendritic Cell Activation and Maturation

Dendritic cells (DCs) are the most potent professional antigen-presenting cells (APCs), efficiently capturing, processing, and presenting antigens. Certain adjuvants promote DC activation and maturation [42,43], thereby enhancing antigen presentation and strengthening immune responses, as seen with oil emulsion adjuvants.

2.6 Activation of Inflammasomes

Inflammasomes are protein complexes approximately 700 kDa in size that constitute essential components of the innate immune system [45]. They regulate caspase-1 activation, promoting cleavage and maturation of pro-IL-1 and pro-IL-18 during innate immune defense [46], and mediate caspase-1-dependent programmed cell death under inflammatory and stress conditions [47], as seen with aluminum adjuvants.

Conclusion

Ideal adjuvants should enhance immune responses with minimal dosage while maintaining high safety without side effects. As adjuvant research continues to advance, future directions must focus on further elucidating mechanisms of action to achieve comprehensive understanding while exploring novel, highly effective, and low-toxicity immune enhancers. Peptide adjuvants, recently identified as immune response enhancers, have demonstrated significant antibody titer enhancement in animal experiments. Derived from probiotic secretion, they are side-effect-free, convenient for transport, and easy to store, making peptide adjuvant research a promising direction for future adjuvant development.

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