Research Progress on the Immunosynergistic Mechanism of New Adjuvants for Recombinant Protein Vaccines

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Abstract: By analyzing the different targets of various types of vaccine adjuvants and traditional aluminum adjuvants, to find an adjuvant that can be biased towards Th1 immune environment to prolong the protection time and protect special groups. The data of new adjuvants that have been successfully used in vaccines were analyzed to compare their safety and reliability. TLR agonists, saponins, emulsions and other adjuvants have significant advantages in the development of long-lasting immunity in special populations.

Keywords: Vaccine, Adjuvant, Safety, Immune Response

1. Introduction

Recombinant protein vaccine is an important form of subunit vaccine. The core technology of recombinant protein vaccine is to introduce and express genes encoding protective antigens of pathogens into specific prokaryotic cells (such as Escherichia coli, yeast) or eukaryotic cells (such as hamster ovary cells), and obtain antigen proteins after recombinant expression and purification.

Examples of commercially available gene engineered subunit vaccines include hepatitis B vaccine and human papillomavirus (HPV) vaccine. However, the immunogenicity of recombinant protein vaccines is weaker than that of attenuated vaccines and inactivated vaccines. Adjuvants are needed to activate the innate immune response, promote lymphocytes to effectively recognize homologous antigens under inflammatory conditions, activate immune cells, and enable the body to obtain protective immunity. Clinically approved vaccine adjuvants for human use include aluminum salt (aluminum adjuvant), MF59 emulsion, and AS series (such as AS01, AS04). So what are the advances in the effects of these new adjuvants in specific vaccines compared with traditional aluminum adjuvants? How is the safety compared with aluminum adjuvants? Can new adjuvants provide higher rates of protection in special populations such as infants, patients with underlying diseases, and the elderly? It is hoped that by elaborating the mechanisms to cross-combine various adjuvants to balance the immune response, optimize the combination of adjuvants, find vaccine adjuvants for special populations, improve safety, reduce vaccine production costs, and meet major safety and health challenges in the future. Based on the immunological basis of recombinant protein vaccines, this review will systematically review the mechanism of action, clinical application and safety of various new adjuvants, and look forward to their future development.

2. Immunological Basis and Adjuvant Requirements of Recombinant Protein Vaccines

After the injection of recombinant protein vaccine, the immune system will produce a specific immune response. The response process is as follows: after the recombinant protein is taken up by the recognition receptor of antigen presenting cell (APC), it breaks down into small fragments and releases cytokines to recruit immune cells to activate adaptive immunity. For T cells: APC is combined with histocompatibility complex (MHC), presented to $CD4^+$ helper T cells, and differentiated into Th1 and Th2 subtypes after TCR recognition and other operations. For B cells: through the surface receptor recognition protein, with the help of various cytokines, proliferation and differentiation into plasma cells, secretion of neutralizing antibodies, thereby blocking the pathogen invasion of cells. After some B cells and T cells are transformed into memory cells, immunity is achieved. The greatest advantage of recombinant protein vaccines is that they are safe, reliable and easy to be produced in large quantities. However, the disadvantage is that the cellular immunity against $CD8^+$T is weak, for example, the antibodies induced by aluminum-

adjuvant HPV vaccine cannot eliminate the existing HPV infection, and at the same time, the effect on tumor cells is poor. Therefore, new adjuvants are needed to solve this problem by inducing a Th1-type immune environment to stimulate a strong $CD8^+$T cell response to those pathogens that are hidden inside the cells.

3. Classification of Novel Adjuvants and Their Immune Mechanisms

The classification of novel adjuvants can be broadly divided into the following categories: Toll-like receptor agonists, including TLR1-10; Saponin-based adjuvants, emulsion adjuvants, nanoparticle/delivery system adjuvants, STING, etc. In this paper, the effects of novel adjuvants that have been successfully applied to recombinant protein vaccines are introduced.

3.1 TLRAgonists

Takumi Kawasaki, Taro Kawai et al. have shown that the innate immune system initiates key responses by recognizing pathogen-associated molecular patterns (PAMPs), which subsequently recruit specific adaptor molecules to activate transcription factors NF-κB and TBK1. Its secretion of IL-12 and IFN-α leads to the generation of Th1-type immune environment, the production of CD80 and CD86 to activate T cells, and B cells to produce longer-lasting memory, which ultimately regulates the final direction of the immune response[1]. These include TLR4, TLR7/8, TLR9 and other types.

3.1.1 TLR4: Common TLR4-type adjuvantsinclude monophosphate lipid A (MPL) and glucopyranolipid A (GLA). TIRAP and TRIF can bind to the adaptor protein MyD88 and activate the NF-κB and MAPK signaling pathways. MPL was developed by Ribi et al., and it is the first TLR ligand approved for use in vaccines. IL-12 p70, CXCL10 and TNF induced by MPL are the key effector molecules, which promote the formation of Th1 immune response and effectively enhance the response[2] against intracellular pathogens. Gla-se is GLA formulated in an oil-in-water emulsion containing squalene, which has been used in clinical trials for malaria, influenza and other vaccines. caspase-1/11 is a prerequisite for IL-18 production. IL-18 can induce NK cells and memory CD8 +T cells to produce IFN-γ, thereby promoting Th1 immunity. At the same time, a large number of neutrophils accumulate at the injection site and draining lymph nodes, and promote cellular immune responses[3] through antigen presentation by dendritic cells.

3.1.2 TLR7/8: Imiquimod (R837), a first-generation TLR7/8 agonist, is a small molecule ofimidazole quinoline, which was initially used as an antiviral agent and later used by the FDA to treat basal cell carcinoma and actinic keratosis. The use ofTLR7/8 agonist as an adjuvant can activate T cell and B cell responses more efficiently and produce longer immune memory[4] . Direct binding to MyD88 is distinct from TLR4, which is a single-pathway endosomal membrane protein. This type of adjuvant plays a significant role in immunodifferential populations. Bharat Biotech et al. developed an inactivated SARS-CoV-2 vaccine (BBV 152) using a conventional aluminum adjuvant and a TLR7/8 agonist as an adjuvant (Algel-IMDG). Th1/Th2 ratio studies revealed a Th1 1-biased cellular response. The incidence of local reactions was similar between the 3μg and 6μg adjuvant groups. The incidence of systemic reactions was higher in the first dose of 6μg adjuvant group, but the seroconversion rate was 81.1% in the 6μg adjuvant group after 104 days,No serious adverse reactions[5] were observed.

3.1.3 TLR9: CpG-ODN, as a nucleic acid adjuvant, activates TLR9 receptors, mimics bacterial CPG-DNA, and activates B cells and dendritic cells to induce immune responses. TLR9 and TLR7/8 regulate the production and secretion of CD40, CD80, IL-6 and TNF-α by activating IRAK and TRAF-6 through MyD88 adaptor protein, thus enhancing cellular immune responses and indirectly enhancing $CD8^+$T cell-mediated cellular immune responses[6]. Based on this property, CPG-ODN can be used as an adjuvant in a variety of ways, including but not limited to muscle, subcutaneous, oral and nasal [7-8]. A TLR9 agonist adjuvant is the now licensed CpG 1018, which is an oligonucleotide that induces Th 1-biased immune responses. In contrast to hepatitis B vaccine with aluminum adjuvant, Th2 humoral immunity is predominantly induced. According to the phase III clinical data, the serum protection rate of HBV-23 vaccine using CpG 1018 as an adjuvant in the age group of 18-29 years, 30-39 years, 40-49 years, 50-59 years, and 60-70 years was 100%, 98.9%, 97.2%, 95.2%, and 91.6%, respectively. The safety of HBV-23 vaccine was similar to that of aluminum-adjuvanted vaccine. The serum protection rates of HBV-23 vaccine

against diabetes and obesity were 90.0% and 94.7%, respectively, and the vaccination completion rate was 5.17 times[9] that of aluminum-adjuvanted vaccine.

3.2 Saponins Adjuvants (SBAs)

Saponins are natural organic compounds with hydrophobic triterpene structures and glycoside chains. Saponins are one of the most promising new adjuvants for enhancing T cell immunity. Most saponins are extracted from the bark of Quillaja saponaria Molina. Inducing the formation of liposomes in $CD11b^{+}$ dendritic cells enhances antigen cross-presentation and activates $CD8^{+}$T cell-mediated cellular immunity, Inhibition of lipid synthesis and knockout of liposome-related genes can block specific T cell activation[10]. In the latest clinical study, Novavax developed a recombinant protein vaccine NVX-CoV2373 against SARS-CoV-2. The adjuvant is Matrix-M, which is composed of saponins combined with cholesterol and phospholipids to form nano-ions. This study showed that the vaccine efficacy in the face of all symptoms of Covid-19 reached 90.4%, and for moderate and severe Covid-19, the vaccine efficacy was 100%. The most common local reactions after 7 days of vaccination were pain at the injection site, and systemic reactions were headache and fatigue, and the two reactions lasted less than 2 days, The experiment tested the Alpha variant of the novel coronavirus, which changes more frequently,It did not test vaccine efficacy against subsequent variants such as Delta and Omicron[11].

3.3 Emulsion Adjuvants

Currently used emulsion adjuvants are O/W emulsions such as MF59, AS03, etc. MF59 is the second FDA-approved adjuvant for human use. The AS series of adjuvants is usually formed by mixing classical adjuvants with immunostimulatory molecules. Both emulsions enhance the efficiency of antigen capture, processing, and surface presentation by a slow-release mechanism. The MF59 emulsion mechanistically induces endogenous danger signals. Intramuscular injection of vaccines containing MF59 adjuvant results in the production of chemokines such as CCL 2, CCL 4, and CCL 5. For the targets and signaling pathways, MF59 emulsion does not activate the NLRP3 receptor like the general aluminum adjuvant, but activates innate immunity through MyD 88 to induce a biased mixed Th1/Th2 type immune response[6] in vivo. AS03 is an emulsion adjuvant composed of squalene, α-tocopherol, PBS buffer, and polyssorbitol 80. As the main component, α-tocopherol is essentially vitamin E, and also has an immune-enhancing effect. The main mechanism is to increase the production of NF-κB transcription induced cytokines, enhance the antigen presentation ability of APC to $CD4^{+}$T cells, and then activate B cells to produce higher titers of antigen-specific antibodies, and induce mainly Th2 response, corresponding to a weak[12] Th1 response. The herpes zoster subunit vaccine (HZ/su) uses AS01B adjuvant, which is composed ofTLR4 agonist MPL and QS21 in combination. In phase 3 clinical studies, the immune efficacy of HZ/ SU in people aged 50-59 years, 60-69 years, and over 70 years was 96.6%, 97.4%, 97.9%, and the overall effective rate was 97.2%.

[13]The RSV PreF3 OA vaccine with different content of MPL and QS21 as adjuvant type AS01E was studied by A. Papi, M.G. Ison et al., and it was found that the preventive efficacy reached 82.6% in the elderly over 60 years old, and the protection of RSV-A/B subtypes was balanced. The protective balance was 84.6% fortypeA, 80.9% for type B, and 94.1% for severe lower respiratory tract infections. [14]These results indicate that AS01 can be used as an adjuvant in elderly patients, and provide a background for the development of vaccines for the elderly.

3.4 Nanoparticle/Delivery System Adjuvant

Nanoparticles are widely used as vaccine adjuvants. Matrix-M, as mentioned above, is an adjuvant that can induce strong Th1-type responses. It is generally configured by a variety of materials, and the surface structure, physical and chemical properties, and hydrophilic/hydrophobic properties can be adjusted. They can be used not only as antigen carriers but also as adjuvants to exert immune effects together. The mechanism is to deliver antigen to the cytoplasm by phagocytosis, activate $CD8^{+}$T through MHC I pathway, and promote cross-presentation, targeting lymph nodes with nanoparticle adjuvant may also eliminate the side effects of other adjuvants, such as the possible systemic effects of TLR7/8 agonists, and studies have shown that it may also have a dose-saving effect[15]. Through the

combination of the above adjuvants, nanoparticles can effectively deliver adjuvants to DCS to enhance their immune responses. Stella Buchhorn de Freitas et al. studied biological silver nanoparticles and found that silver nanoparticles induced both cellular and humoral immunity with a lower risk of inflammation than aluminum adjuvants, inducing only IL-10 but not TNF-α ,the synthesis of bio-silver nanoparticles by biosynthetic method is more green and environmentally friendly[16].

4. Clinical Application and Safety

Based on the above data, it can be analyzed that new adjuvants have better immune efficiency in different populations or diseases. As for TLR7/8 agonists, the vaccine with 3μgTLR7/8 agonist and 6μgTLR7/8 agonist had similar safety profile and could induce more durable humoral and cellular immune responses after the second dose. For TLR9 agonists: In general, the protection rate of hepatitis B vaccine with aluminum adjuvant begins to decline at the age of 40 years, and it is about 75% by the age of 60 years, and only one third of the population has completed the full vaccination. HepB-CpG has shortened the vaccination cycle and greatly improved the patient compliance. The protective rate of aluminum adjuvant against diabetes, obesity, and the elderly (60-70 years old) was only 65.1% and 75.4%, respectively. 72.6%, much lower than CpG 1018; Saponins: Saponins prevented moderate or severe Covid-19 after vaccination, and no moderate-to-severe cases occurred, while placebo had 18 moderate-to-severe cases. The safety was good, and the common adverse reactions were mild or local reactions. For the AS01B adjuvant: Compared with the live attenuated vaccine Zostavax, the immune effect in the elderly is significantly increased, which well eliminates the problem of decreased immune effect in the elderly. At the same time, the safety is good, and there is no toxicity reversion problem caused by the live attenuated vaccine. As01e has a significant protective effect for severe diseases, and has good safety.

5. Challenges and Prospects

Although the types of new adjuvants are constantly expanding, safety is always the first research priority. For example, STING (Stimulator of interferon genes), which is also a new type of immune adjuvant, has not passed phase III clinical trials as a comprehensive adjuvant that can be used in cancer, HIV, and anti-parasitic treatment. It is not that this kind of adjuvant has defects in the target, but the therapeutic window is extremely narrow, and high fever, hypotension, and acute respiratory distress syndrome may occur after use. The adverse reactions are much greater than those of aluminum adjuvant, and its toxicity is far beyond the acceptable range[17] of normal people.

In terms of reactogenicity, there may be local adverse reactions including pain and redness at the injection site, systemic symptoms such as fatigue and fever. Studies have shown that the reactogenicity profile of any adjuvanted vaccine varies[18] depending on the antigen being studied and the target population, so it is important to pay attention.

For special populations such as infants, young children, and the elderly, to ensure the persistence of immune responses and antibodies, risk monitoring should be continuously carried out when new adjuvants are used. More comprehensive safety and efficacy data are needed for the approval of new adjuvants in the regulatory path. In this regard, there may be a future design of vaccine adjuvants for special populations by optimizing the technology to produce nanoparticles or their delivery systems to achieve synergistic effect, and may reduce the dose of single components to reduce the side effects. Reasonable selection of adjuvants and antigens can better protect people with different immune responses. In terms of mechanism research, although aluminum adjuvant is the only adjuvant used in human body 97 years ago, the mechanism of action of this adjuvant has not been elucidated, and its activation pathway is controversial[19] .

6. Conclusion

Aluminum adjuvant, as a successful adjuvant, has been used on a large scale, but it tends to form Th2 type immune response, $CD8^{+}$T cells are not differentiated enough, and the ability to clear infected cells is poor, and it cannot destroy the pathogens hiding in the cells. New adjuvants such as TLR7/8 can balance the Th1/Th2 response when combined with aluminum adjuvants, TLR9 CpG-ODN has a higher protection rate for people with special diseases, saponins have advantages in coping with major infectious diseases, and emulsion adjuvants AS01 series have a better response rate

especially for the elderly. Adverse reactions can be reduced by nanoparticle delivery technology. The immune response induced by nanoparticle delivery is similar to that induced by aluminum adjuvant, but the production is more environmentally friendly and economical, and will also reduce the occurrence of inflammation. Due to the particularity of the new adjuvant mechanism, it is possible to produce tumor therapeutic vaccines in the future.

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