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ORIGINAL REVIEW

Current Strategies to Improve Efficiency of Recombinant Subunit Vaccines

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Abstract

Global population growth and rising health concerns have highlighted the importance of developing effective vaccination strategies, and subunit vaccines hold great promise for meeting market demand. Compared to traditional whole-cell vaccines, subunit vaccines contain only a part of the infectious microorganism to induce an immune response against the main pathogen. Recent advances in molecular biology have paved the way for the development of recombinant subunit vaccines to provide ideal vaccination strategies. The production of these vaccines is a multi-step process in which each stage should be thoroughly evaluated with the goal of improving vaccine efficiency while remaining cost-effective. In this review, different approaches used for the improvement of recombinant subunit vaccines are described, considering production steps and vaccine formulations. Firstly, a brief summary is presented about different types of vaccines, especially recombinant subunit vaccines, and then several strategies, including novel expression systems, the selection of suitable adjuvants, and the integration of nanotechnology into the vaccine formulations are detailed in the light of the most recent progress. This article highlights the critical aspects of the development of more effective recombinant subunit vaccines with broad-spectrum protection.

Keywords

Recombinant antigen, *Escherichia coli, Chlamydomonas reinhardtii*, Malaria, Hepatitis, Adjuvants, Vaccine, Nano-technology

Introduction

During the past few years, an emerging global threat, called Coronavirus Disease 2019 (COVID-19), has become a major health problem all around the world, and highlights the importance of immunization with vaccination. Vaccines are considered the most promising fighters against viral infections like COVID-19, hepatitis, influenza, smallpox, malaria, diphtheria, etc. According to reports from the World Health Organization (WHO), the deaths of nearly 3 million people have been prevented every year by developed vaccines [1,2]. Besides health impacts, vaccination has an important economic benefit by saving over $500 billion in medical expenses compared to scenarios without vaccines [3]. Looking at the history of vaccination, several diseases such as smallpox, polio, and measles have been eradicated or controlled via mass immunization [4]. However, there are still many health threats that scientists and authorities have been exhibiting great effort to control them via different types of vaccines and immunization strategies. These efforts have focused on both the development of new vaccination approaches or improvement of the effectiveness of existing vaccines [5].

For more than two centuries, scientific studies have resulted in the development of a wide variety of vaccines which can be divided into different categories based on the nature of the vaccine antigens or production technology [6]. Live attenuated vaccines are one of the most common conventional vaccine types that induce a strong immune response against many infections. They contain live or modified pathogenic agents attenuated by adaptation to low temperatures or by performing serial passage through *in vitro* cultures, live animals, or embryonated eggs. Looking at history, many of the first vaccines against common diseases such as rabies, measles, polio, smallpox, and yellow fever were in type

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of live attenuated [7,8]. In spite of their high efficiency, the possibility of the viruses regaining their toxicity due to mutations after immunization may limit the use of this vaccine [9]. Killed or inactivated vaccines are considered safe and stable alternatives since they contain nonliving pathogens that are destroyed by applying heat, radiation, or chemical agents. To date, these vaccines have been developed successfully to protect against the devastation caused by hepatitis A, rabies, influenza, and polio viruses. However, one major obstacle to inactivated vaccines is that they possess a weak immunogenic effect and provide inadequate long-term protection, thus requiring multiple booster doses [10,11]. Toxoid vaccines, another type of vaccination approach, contain inactivated forms of toxins secreted by pathogens. The development of these vaccines involves the elimination of the harmful effects of native toxins by applying heat treatment or chemicals (mostly formaldehyde), resulting in the formation of toxoids. Normally, native toxins lead to the production of many symptoms and cause disease after infection with pathogens, but toxoids induce an immune response in a safe and efficient way by taking part in vaccine formulations. The most popular examples of toxoid vaccines are tetanus, diphtheria, and pertussis [12,13]. Recent progress made in biotechnology and immunology has shifted the focus of vaccinology strategies to the development of recombinant subunit vaccines. Traditional subunit vaccines, which contain a part of the infectious microorganism rather than the whole pathogen, have already been used against several diseases like pertussis or influenza. However, these vaccines have encountered some disadvantages during commercialization, including low efficiency, biosafety issues, cost-effectiveness, and complexity of scale-up [14,15]. At this point, recombinant subunit vaccines allow for the development of vaccines against a wide range of viruses while eliminating the challenges associated with traditional subunit vaccines through the use of more controlled bioprocess in a shorter production time. This process involves the transfer of a gene encoding an antigen to the different heterologous hosts, like yeast, bacterial species, insects, and mammalian cells [16,17]. A typical example of recombinant subunit vaccine, which is approved for use in humans, was developed against hepatitis B by expressing the surface antigens of the virus in yeast cells. Also, the human papillomavirus vaccine is another recombinant subunit vaccine that is currently commercialized worldwide. The high efficacy of these vaccines has been proven by authorities, and more potential recombinant subunit vaccines are on the way to development and approval [18,19]. Preparation of an effective subunit vaccine faces critical issues of low immunogenicity, short half-life, and strain-specific protection. Thus, researchers have tremendously focused on solving these challenges, and much more study should be conducted for further progress [15,17].

In this review, current strategies to eliminate the problems of recombinant subunit vaccines and improve their efficiencies are summarized. Firstly, a brief summary is presented for the development of recombinant subunit vaccines, and then several strategies, including novel expression systems, the selection of suitable adjuvants, and the integration of nanotechnology into the vaccine formulations are detailed in the light of the most recent progress. The review included English-language articles published in high-quality, peer-reviewed journals. The bibliographic review was conducted according to three databases: Web of Science, Science Direct, and Scopus.

Recombinant Subunit Vaccines

The fundamental idea behind a subunit vaccine is based on the isolation and transfer of the gene encoding the immunogenic components to another non-pathogenic organism. At this point, it is critical to obtain adequate information about the sequence of the gene related to the target antigen and to design and synthesize suitable gene expression constructs. To amplify the desired coding sequence, polymerase chain reaction (PCR) method is usually performed that enables the isolation and purification of the gene region [17].

The constructed expression vectors are then transferred to host organisms considered production platforms for target components. The selection of suitable hosts depends on several factors, such as the level of expression, the need for post-translational modifications, the stability of the final product, and the total cost of the process (Table 1). Bacterial

Table 1: Advantages and disadvantages of different host expression systems [14,20,22].

Bacteria	Yeast	Insect	Mammalian
✓High productivity ✓Fast growth rate ✓Cost-efficient ✓Easy to manipulate genetically	✓High productivity ✓Fast growth rate ✓Cost-efficient ✓Possibility for post-translational modification ✓Bio safe	✓High level of protein production ✓Possibility for post-translational modification ✓Chance of studying with multiple genes	✓Ability to make complex post-translational modifications ✓Secretion of molecules in serum-free medium
×No post-translational modifications × Generation of contaminant endotoxins	× Distinctive glycosylation pattern	× Distinctive glycosylation pattern × Risk of virus infection × Moderately higher cost	× Low expression level × Expensive to cultivate

cells, especially *Escherichia coli*, are one of the most common types of expression systems due to their high productivity, significantly lower production costs, and possibility of genetic manipulation. Despite these benefits, the use of bacterial systems has been hampered by the lack of eukaryotic post-translational modification, which has resulted in the production of misfolded and nonfunctional proteins. To overcome this challenge, genetically engineered cells can be used, which provide expression of proteins properly and also may enhance the specific properties of proteins such as solubility, efficiency, and stability. However, genetic modifications increase the complexity and cost of the process [20-22]. As another expression system, yeasts are highly preferred for subunit vaccine production. In addition to the advantages specified for bacterial systems, yeasts have the ability to perform post-translational modifications in a similar way to the higher eukaryotic cells. However, expressed proteins are glycosylated with a different pattern from those in mammalian cells, which may cause the excessive level of glycosylation. *Saccharomyces cerevisiae* and *Pichia pastoris* are the most extensively used yeasts for vaccine production, and various subunit vaccines against hepatitis, influenza, and papillomavirus have been developed by using these organisms [14,17]. The insect cell-baculovirus expression system is a novel and efficient approach for recombinant protein expression. These cells enable a high level of protein production with an appropriate post-translational modification step, but their glycobiology differs from mammalian cells as in yeasts. To date, insect expression systems have been commercially used for the development of veterinary vaccines, such as those against the swine fever virus and the porcine circovirus [22,23]. Mammalian cells are perhaps an optimal choice for recombinant subunit vaccines, given their significant success in making precise post-translational modifications. However, they have a low production rate, and their total cost is much higher than other expression systems. Nevertheless, Chinese hamster ovary (CHO) cells are most frequently used cell line considering their high protein efficiency and safety. There are many ongoing studies that aimed to development of subunit vaccines using CHO-based systems, and some of them presented several successful vaccines against hepatitis B, cytomegalovirus, and varicella zoster virus [14,20].

Strategies to Improve Recombinant Subunit Vaccines

Until today, recombinant technologies have enabled the development of a wide variety of vaccines, but there are still concerns about the existing vaccines, and novel vaccination strategies are required for many diseases. In order to overcome the problems mentioned above and develop new efficient vaccines, much research has been conducted by scientists considering the production technologies and vaccine formulations.

One of the substantial strategies in vaccine improvement is the use of novel alternative expression systems instead of traditional ones (bacteria, yeast, insect, and mammalian). With the recent developments in plant biotechnology and immunology, studies have focused on the use of different plant species as bioreactors for recombinant protein production. Plants offer an attractive alternative to the common expression systems since they possess several advantages, including cost-effectiveness, ease of scale-up, ability to perform post-translational modifications similar to mammalian cells, and great bio safety due to the lack of infectious pathogens. Besides, the expression of immunogenic components in edible plants allows the vaccine to be delivered orally, which makes the vaccination approach simple and safer. Although protein expression systems in plants may initially seem attractive, a few obstacles need to be solved before they can be extensively used. Some of the issues are associated with the use of genetically modified plants and regulatory approvals because authorities have concerns about the spread of recombinant genes among other species and the possible hazardous effects of these plants on humans after consumption. Therefore, there should be well-established regulatory criteria for growth and use of transgenic plants in recombinant vaccine development [24-26]. Transgenic animals, another platform for the production of subunit vaccines, can generate large amounts of high-quality proteins at a relatively low cost. Some of the possible expression systems of transgenic animals are milk, blood, egg white, urine, seminal plasma, and silk gland. Each of them has their own pros and cons, but milk is presently considered the most efficient mature platform due to its high production levels, easy accessibility, and practicality for commercialization [27,28]. For example, Li, et al. [29] investigated the development of a recombinant subunit vaccine against rotavirus and used the milk of transgenic mice for the expression of the main component of viral structure protein. They reported on the viability of milk as an expression system and suggested alternative sources of milk supply, such as goats or cows. Recently, microalgae species, especially green microalgae, have been of great interest as expression systems for obtaining recombinant components. These cells have a high growth rate and can be easily cultivated in open ponds or closed photo bioreactors under optimum conditions [30]. Also, they are able to perform correct post-translational modifications and properly fold complex proteins. Among numerous species, *Chlamydomonas reinhardtii* has been mainly used to produce recombinant therapeutics because the nuclear, mitochondrial, and chloroplast genomes of these cells have been sequenced and many genetic engineering tools are available for this strain. Several studies have reported the successful application of *C. reinhardtii* cells for the development of vaccines against Newcastle disease [31], human papillomavirus [32], classical swine

fever virus [33], and malaria [34]. *Phaeodactylum tricornutum*, *Schizochytrium* sp., *Thalassiosira pseudonana*, *Nannochloropsis* sp., and *Chlorella* sp. are some of the other microalgae species that have proven to possess strong potential for recombinant vaccine production. On the other hand, expression in these organisms may result in an improper glycosylation pattern and a low level of protein. Thus, much more studies are required to replace the traditional expression systems with microalgae species [35,36].

During vaccine development, one of the key components is adjuvants, which play an important role in enhancing and guiding the adaptive immune response to vaccine antigens. In other words, adjuvants promote a rapid immune response, produce long-lasting memory, and lower the amount of antigen that is needed for a successful immunity [37]. Various adjuvants, including aluminum salts, squalene oil-in-water (O/W) emulsions, liposomes, and polysaccharides, have been used in vaccine formulations to enhance efficiency. The low immunogenicity of recombinant subunit vaccines is a critical problem that can be solved through the addition of different types of adjuvants. Traditional vaccine formulations with a single adjuvant generally induce an acceptable level of immune response, but studies indicate that the combination of some adjuvants may improve the vaccine's effectiveness [37,38]. Currently, significant effort has been expended toward the development of more efficient vaccines against malaria, and combinations of adjuvants have been evaluated in many studies. Mordmüller, et al. [39] aimed at the development a malaria vaccine using the *Drosophila* cell line as expression system and tested different adjuvant combinations of glucopyranosyl lipid adjuvant (GLA), squalene-based O/W emulsions, and liposome formulation of QS-21 adjuvant. They reported that the vaccines formulated with GLA-based adjuvants had higher activity than those involving single adjuvant system. In another study, a subunit malaria vaccine against *Plasmodium vivax* was developed by expressing proteins in *E. coli*, and the produced protein was delivered in three adjuvants: naloxone, CpG oligodeoxynucleotides, and 3-O-deacylated monophosphoryl lipid A, individually and in combination. The combination of three adjuvants led to an increase in the antibody levels and an enhancement of retention time [40]. These outcomes were supported by similar studies, and the combination of adjuvants holds great promise for improving recombinant subunit vaccines [41]. Another interesting progress made in the development of the malaria vaccine is improving the solubility of the interested protein during its expression by using a second protein as a solubility tag. According to the literature, a second granule lattice protein (Grl3p) from *Tetrahymena thermophila* improves the protein solubility using the expression of *Plasmodium* antigens in *E. coli* [42,43].

The field of nanotechnology can be implemented into vaccine development research to enhance the immune response. Nanoparticles, which are organized structures on a nanoscale of 1-100 nm, can be utilized as vaccine carriers to preserve the original conformation of antigens, protect them from degradation, and provide prolonged exposure due to slow release of components. The effect of a nanoparticle on vaccine activity depends on the size, conformational structure, and surface charge of the particles [44]. Nanoparticles can be synthesized chemically or derived from biological sources. Some of the biologically-derived nanoparticles are virus like particles, outer membrane vesicles, ferritin cages, encapsulins, virosomes, and liposomes. Among them, virus like particles have attracted much more attention since they easily mimic the parent virus without causing any infection. Today, several vaccines containing virus like particles against the human papillomavirus and hepatitis B have been approved by the Food and Drug Administration (FDA). Also, ferritin cages, which are protein assemblies derived from ferritin, have a strong potential to be integrated into vaccine formulations due to their versatility, but their rigid assembly limits the use of these particles [20,45]. The practical application of biologically-derived nanoparticles as vaccination platforms improved the efficacy of recombinant subunit vaccines, while there are more options to be evaluated for the development of an ideal vaccine strategy.

Conclusion

Recombinant subunit vaccines are some of the most effective and safe vaccines, and novel approaches are of great interest to develop new and improved vaccination strategies. Traditional recombinant technologies use bacteria, yeast, insect, and mammalian cells as expression systems, but recent progress has pointed out some alternative platforms, including plants, transgenic animals, and microalgae species, which show superior potential to clone interested genes. Among them, plants and microalgae are highly promising hosts due to proper expression of antigens and biosafety. Another approach to vaccine improvement is the use of adjuvants in combinations rather than the addition of single adjuvants to the formulations. Research has proven that combined adjuvants provide a higher antibody level and longer-lasting immunization than single adjuvant systems. Finally, the integration of different nanoparticles like virus like particles, ferritin cages, and outer membrane vesicles into the vaccine formulations is an efficient way to preserve the structure of antigens and protect them from degradation. These approaches have the potential to improve the efficiency of recombinant subunit vaccines, but more studies are needed to replace traditional vaccines with these improved alternatives.

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