Vaccination under COVID-19 Pandemic

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The current COVID-19 pandemic, caused by severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2), has raised significant economic, social, and psychological concerns. The rapid spread of the virus, coupled with the absence of vaccines and antiviral treatments for SARS-CoV-2, has galvanized a major global endeavor to develop effective vaccines. Within a matter of just a few months of the initial outbreak, research teams worldwide, adopting a range of different strategies, embarked on a quest to develop effective vaccine that could be effectively used to suppress this virulent pathogen.

Keywords: vaccine adjuvant ; viral vector ; DNA vaccine ; RNA vaccine ; nanoparticle

1. Introduction

The outbreak of the COVID-19 pandemic[1][2][3][4], which was caused by severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2), has triggered a global race to develop effective vaccines. Approximately 150 different research groups are currently involved, and more than 100 clinical trials have been initiated since the outbreak was first reported[5]. They all have the singular goal of developing and producing an antiviral vaccine that is effective in individuals of all age groups with all conditions, and, thereby, control the course of the pandemic. Nevertheless, the development of a vaccine is a laborious process, the mass production, distribution, and administration of which present extraordinary challenges, particularly in developing countries. Accordingly, the strategy that has been employed for vaccine production needs not only to take into consideration the effect of the vaccine on the immune system and its efficacy against the virus, but also the procedures for mass production, distribution, storage, and mass vaccination[5]. To this end, the participating research groups are employing a diverse range of different formulations, techniques, and strategies to produce effective vaccines against SARS-CoV-2. In this regard, there are four main methods of vaccine development, namely, employing pathogens (inactivated or with low virulence) for the production of vaccines; recombinant protein vaccines; vector-based vaccines that include DNA vectors or viral vectors; and, the latest technology using RNA molecules for vaccination. Among these, the more innovatory next-generation vaccines only use a part of the virus protein structure and, thus, can be expected to have a superior safety profile. However, these novel vaccines tend to have low immunogenicity and they often fail to induce a sufficient immune response. Consequently, we also describe the use different adjuvants, which can be employed in order to enhance immunogenicity and establish an enduring immune memory.

2. Next-Generation Vaccines

In 1990, Wolf et al. demonstrated that mice injected with plasmids harboring a cloned protein subsequently showed an expression of the transgenic protein cloned in the plasmid DNA[6]. These observations provided an impetus for the development of a new strategy of vaccination, and marked the advent of an era of next-generation vaccines. The initial strategy adopted for these novel vaccines was a DNA-based technique, which was subsequently followed by the development of viral vectors, including adeno-associated virus (AAV), lentiviral, or adenoviral vectors for vaccination, and, more recently, by RNA-based vaccines. The salient point of this research is that it demonstrates that only a portion of the viral protein structure is sufficient for promoting immunity against a given pathogen. Consequently, these innovatory vaccines tend to only include a specific viral antigen, instead of employing the entire pathogen, thereby resulting in a better safety profile[7]. However, the design of such vaccines requires a more in-depth understanding of viral structures and the interaction between viral proteins and host cell receptors, and, accordingly, these next-generation vaccines tend to require a lengthy phase of preliminary studies before development can commence.

2.1. Recombinant Protein Vaccines

Recombinant protein vaccines are based on the use of recombinant viral structural proteins to induce an immune response. In this respect, the SARS-CoV-2 genome comprises four structural proteins, namely, membrane, envelope, nucleocapsid, and spike proteins. Among these, the spike protein is of particular importance, given that it interacts with

angiotensin-converting enzyme 2 (ACE2) receptors that are localized on the surface of host cells, thereby facilitating endocytosis[8]. Consequently, most vaccination strategies for the SARS-CoV-2 virus have focused on this protein, owing to its importance in the virus lifecycle. However, vaccination with whole spike protein has been shown to promote liver damage in treated animals[9] and, thus, the use of only a part of this protein, such as the receptor-binding domain (RBD), which interacts with the ACE2 receptor protein, is considered to be the best alternative with respect to producing a safer vaccine [10]. Initial research in this regard suggests that immunization with recombinant protein or only the RBD results in the production of neutralizing antibodies[10][11][12]. Observations indicated that the protein is processed by dendritic cells, followed by the presentation of the antigen to naïve B and T cells, resulting in their activation and subsequent immunity development. However, the use of this strategy for immunization has a notably important drawback, namely, that, owing to the use of only a small part of the protein for immunization, specific immune reactions induced by the vaccine confer only partial protection[12][13][14]. Moreover, these immune reactions tend not to be particularly strong[15]. Consequently, vaccination with recombinant proteins necessitates the use of substrates, referred to as adjuvants, to boost the immune response. The use of such adjuvants enhances antigen presentation in antigen-presenting cells (APCs), thereby enhancing vaccine efficacy and resulting in long-term protection.

2.2. Plasmid DNA Vaccines

Wolff et al. demonstrated that intramuscular injection of nucleic acids resulted in the in vivo expression of a protein encoded by plasmid DNA[6], and it was later shown that vaccination with plasmid DNA can induce a strong immune response, as mentioned previously[16][17][18]. Collectively, the findings of these studies have provided evidence of the potential of plasmid DNA to produce immunization on injection. Subsequently, researchers began to examine the utility of DNA vaccines for the treatment of cancer, infections, and autoimmune diseases, including allergies [19]. However, the early-stage clinical studies in humans tended to be unsuccessful, owing to the poor transfection efficacy and low immunogenicity. Nevertheless, DNA vaccines do offer certain advantages[19]. First, the use of plasmid DNA for vaccination is safer than certain traditional vaccines, in that it avoids the administration of a live virus. Second, plasmid DNAs tend to be more stable than proteins, viruses, or mRNAs, and they can be freeze-dried and maintained in long-term storage. Third, the production of these vaccines is more straightforward and cost-effective. In recent years, improved transfection methods, such as electroporation based on the use of electric pulses to perforate the cell membrane, have been developed in order to enhance plasmid transfer into cells. The use of adjuvants to boost the immune reaction has been further advance in the development of DNA vaccines, which has increased the suitability of DNA vaccines as an ideal type of vaccine for mass administration. In this context, the company Inovio performed one of the earliest vaccination studies targeting the MERS coronavirus in order to develop a new DNA vaccine for COVID-19 [20]. Immunization with the synthetic DNA-based vaccine (INO-4800) targeting the SARS-CoV-2 spike protein resulted in the strong expression of this protein, and it promoted antigen-specific T cell responses and the production of antibodies, which were able to bind to ACE receptors and neutralize SARS-CoV-2 infection [20]. Previously, Inovio had also developed similar DNA vaccines against the Ebola[21], SARS [22], MERS [23][24], and Zika [25] viruses. Other previous studies have similarly used DNA-based vaccines to generate immunity against *Toxoplasma gondii* in mice[18], and also to produce a T-cell-dependent antibody response to glutamic acid decarboxylase[17].

2.3. Viral Vector Vaccines

Although the use viral vectors for therapeutic purposes commenced in the late 1990s, the application of these vectors for disease treatment was primarily overshadowed by the death of Jesse Gelsinger, who was administered an adenoviral vector [26], as well as the development of leukemia in children with severe combined immunodeficiency (SCID) treated with retroviral vectors [27][28]. However, in recent years, significant progress in the development of viral vector vaccines has yielded encouraging results with respect to dendritic cells, and an increasing number of studies have begun to focus on the use of different viral vectors, including RNA (retroviral and lentiviral), adenoviral, and Adeno-associated virus (AAV) vectors [29][30][31][32]. Immunization based on viral vector vaccines entails cloning the immunogenicity-causing antigen in a pseudovirus, which lacks the ability to propagate and transfer in dendritic cells, thereby producing stronger immune stimulation than recombinant proteins[33].

2.4. RNA-Based Vaccines and Nanoparticle (NP) Formulations

RNA-based vaccines are the most recent development in the quest to produce safe and efficacious means of vaccination. One of the major factors that has hitherto prohibited the use of RNA for vaccination is its low stability. Furthermore, RNA only enables transient expression and it is negatively charged and, consequently, the use of additional substrates is necessary for facilitating the entry of RNA into cells. However, recently, different strategies have been developed to enhance mRNA stability and the delivery of RNA into cells, which have contributed to making RNA-based strategies among the most efficient methods of vaccination.

2.5. Vaccine Adjuvants

Vaccines have been extensively established as powerful tools in combating diverse diseases. Traditional vaccines, including the use of inactivated pathogens or pathogens with reduced virulence, are characterized by the induction of strong immunogenicity, low production costs, and relatively straightforward preparation processes. However, generally, they tend to have poor safety profiles[34], which has led to the emergence of alternative next-generation vaccines, including recombinant protein vaccines, DNA-, virus-, and RNA-based vaccines with better safety profiles. However, these novel vaccines, particularly those employing RNA, plasmids, and recombinant proteins, are typically characterized by low immunogenicity[15]. Consequently, there is an urgent need to develop adjuvants that can be used in order to enhance the immune reaction and increase vaccine efficacy. Adjuvants enhance antigen presentation in antigen-presenting cells (APCs), thereby improving immunogenicity and ensuring long-term protection. As long ago as 1930, aluminum adjuvants were first used in clinical trials and they are still used in approximately 80% of those vaccines delivered in adjuvants [35]. Aluminum adjuvants can stimulate the immune system via different pathways, and they have been shown to bind to and alter the membrane structure of dendritic cells[36]. Moreover, they may either induce apoptosis or stimulate NLRP3 inflammasomes in order to produce threat signals, thereby initiating an immune reaction[37][38]. However, as the use of aluminum adjuvants can be associated with the induction of weak cellular immunity and they are ineffective against intracellular viral infection[39], a new type of adjuvant containing monophosphoryl lipid A and aluminum hydroxide has been developed for vaccines for hepatitis B and papillomaviruses[40]. Similarly, a combination of aluminum and CpG has been used against malaria[41], and nano-aluminum adjuvants [42] have also been employed. Furthermore, Jiang et al. developed PEG-coated nano-aluminum particles that could enter lymph nodes and showed synergistic effects with CpG[43]. Recently, different companies have developed emulsion adjuvants, being classified as oil-in-water emulsion adjuvants, including AF03, MF59, AS02, and AS03[44][45][46], or water-in-oil emulsions, including Montanide ISA51 and ISA720[47][48]. These emulsion adjuvants can be used to induce high humoral immunity via different interactions. For example, in the case of MF59, this effect is attributable to the induction of threat signal release from muscle cells at the injection site. Furthermore, the effect was found to be associated with apoptosis-related speck-like proteins (ASC) containing a caspase recruitment domain, and the activation of the MyD88 gene[49]. More recently, Xia et al. coated a core comprising a mixture of squalene and all-*trans* retinoic acid with a shell of poly(lactic-*co*-glycolic acid), which was found to enhance the expression of CCR9 on the surface of dendritic cells, resulting in antigen uptake, homing of these cells in the lymph nodes, and, consequently, the induction of strong mucosal immunity[50].

AS01, which is used as an adjuvant with vaccines for herpes zoster and malaria, is an adjuvant system of particular interest. This preparation is based on liposomes that are derived from cholesterol in combination with dioleoylphosphatidyl-choline and two immunostimulants, namely, QS21 (purified saponin) and MPL (a derivative of lipopolysaccharide), which have a synergistic effect[51][52]. Although QS21 is potentially toxic, cholesterol reduces this toxicity, thereby improving the safety of the adjuvant. After administration, QS21 translocates to the lymph nodes, wherein it accumulates and stimulates caspase-1, which is followed by the production of high-mobility group protein B1 and activation of the TLR4-MyD88-related pathway[53]. A further adjuvant derived from AS01 is AS015, which, combined with CpG oligodeoxynucleotide 7909, has been used in conjunction with a vaccine for melanoma[54][55], and it can also enhance anti-cancer activity [56][57]. Other researchers have used poly(lactic-*co*-glycolic acid)] or natural chitosan, which have good safety and biocompatibility profiles, to protect antigens and enhance antigen uptake by APCs[50][58]. Chitosan adjuvants comprise particles of differing forms, sizes, pH values, and surface charges. In the case of acid-soluble chitosan adjuvants, following uptake by APCs, the particles are solubilized in lysosomes, thereby promoting changes in lysosome pH and conformation and, consequently, the release and expression of the antigen. Subsequent to degradation, APCs present the antigen to naïve T cells, which are accordingly activated[58].

Protein adjuvants are the final types of adjuvant described in this review, which include heat shock protein (HSP), GM-CSF, flagellin, and cytokine (e.g., IL2)-based preparations. Protein adjuvants are delivered and expressed as a single protein in combination with the antigen and they are characterized by a good safety profile. Moreover, the findings of previous studies have indicated that co-delivery of the antigen with these adjuvants can significantly strengthen the immune reaction [59][60][61].

3. Conclusions

The use of vaccines can be traced back to the 18th century, when diseases, such as smallpox, were successfully treated while using pathogens with reduced virulence. Since that time, vaccination strategies have undergone a continual evolution and a number of different vaccine types have been used to treat diseases that are caused by a diverse range of pathogens, as well as in combatting cancer. In addition to the more traditional methods of vaccination, there is an ongoing emergence of new-generation technologies, including viral vector-based techniques and RNA-based vaccines. Progress

in the development of each of these novel vaccine types has had to contend with multiple challenges, not only with respect to the underlying scientific concepts, but also in terms of the logistics of mass production, distribution, storage, and mass vaccination. During the development of vaccines for the treatment of Covid-19, the efficacy of all strategies developed thus far has been assessed. On the basis of present evidence, it can be concluded that the RNA-based vaccines are probably superior with respect the timescale of development; however, the associated costs tend to be higher than those of other strategies, due to the necessary specifications of production, distribution, and storage.

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