

Virus Like Particle Vaccines Classification Based on Source Virus

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Abstract. Virus like particle is a self-assembled structure derived from viruses without replicating genome. Thanks to the multiple epitopes at the surface, virus like particles can induce immune response efficiently and thus, being widely used in the vaccination field. Virus like particle vaccination nowadays covers a range of diseases, such as infectious disease, cancer, allergy, and even cancer. In this literature, the author is going to introduce the background and features of virus like particles. Then, the virus like particles are classified based on the classes of virus source. Representatives of commercialized vaccines in different classes are introduced. Discussions are made about the advantages of and the challenges faced by virus like particle fields.

Keywords: Virus like particle; vaccine, capsid; envelope; human papillomaviruses.

1. Introduction

In the 21st century, vaccination plays a significant role in health, as it prevents an average of six million deaths a year and reduces the health cost by 16-fold [1]. Also, they were designed to fight against different pathogens such as viruses, bacteria and even cancer cells [2]. In 19th century, a smallpox vaccine was invented by Jenner to fight against cowpox, which consists of heterologous virus coming from infected cows [3]. Then, toxoid vaccine is invented to fight against rabies, cholera, polio and other diseases [4]. It is featured with the inactivation of bacteria or viruses by applying high temperature or chemicals. In the 21st century, thanks to the development of recombinant technologies, scientists can genetically engineer the virus and produce live and attenuated viruses, which were later called viruses like particles [5].

Hepatitis B vaccine is the product of the recombinant technologies. The hepatitis B vaccine is one of the products of those virus like particle vaccine. The vaccine consists of a cloned surface antigen, HBsAg, coming from HBV. Since the cloned subunit is not infectious and induces immune response effectively, it replaces the previous heat killed vaccine [6]. The design principle of HBV vaccines stimulates the development of VLP vaccine, encouraging more virus vaccines to come into the public sight. Cervarix and Gardasil were designed to fight against cervical cancer [7]. In the middle of 20th century, the first plant vaccine was developed from the tobacco mosaic virus (TMV) to fight against poliovirus in rats. It was designed with TMV CP gene and an antigenic peptide from poliovirus. The purified virus like particles from the bacteria cell stimulates the immunogenic response effectively to eliminate poliovirus [8].

Compared to other vaccines, virus vaccines have ample of univities which make them stand out. Naturally, viruses are naturally selected to have optimal size to go through the pores in lymph vessel and get into the lymphatic system [9]. This improves their chances of being captured by antigen-presenting cells (APC) [9]. Also, thousands of antigens are at the surface of viruses. They are repeated units for antibodies to recognize in the mammalian body. These antibodies then stimulate B cells to fight against any foreign bodies similar to the repeated units [9].

Although many research and experiments have been conducted and made on virus like particles vaccine, there is a few literatures that integrates and classifies the virus like particles based on different virus sources. To this end, this review aims to present a brief summary of the definition of virus like particles, their unique features, and classification of several virus like particle vaccines

derived from human, animal, and plant viruses. In the end, the current challenges faced by the virus like particle vaccine field will be discussed.

2. Virus like particle vaccine

2.1. Virus like particle vaccine feature

Virus like particles vaccines is capsid or envelop proteins made from viruses without viral genomes. Since viruses like particles lack replicating genome from the virus leading to the fact that they cannot self-replicate, they are considered safe [10]. These proteins include coat proteins, structural proteins and envelope proteins, which self-assemble spontaneously. Although viruses like particles have similar structures, they vary in sizes, which can range from 20 to 200 nm, which are favorable sizes to go through lymphatic nodes and getting uptake by APC [10]. They also have different geometries, but most of them resemble icosahedral structures and rod shapes [11]. Moreover, virus like particles have ordered and repetitive epitopes, which can be recognized by the antibodies and the stimulation of APC. Therefore, viruses like particles can easily stimulate the formation of T helper cells, which produce cytokines that is responsible for other immune cells such as macrophages, B cells and T cells [11].

2.2. Virus like particle structure

Based on the structure of coat proteins, virus like particles can be classified in three classes: naked virus like particles, enveloped virus like particles, and chimeric virus like particles. Naked virus like particles are particles without membrane. Its surface is solely composed of proteins. These virus like particles usually have higher susceptibility to the environment such as temperature, pH, and chemicals [12]. Enveloped virus like particles are particles with membrane, which require host's membrane for development. Compared to naked virus like particles, enveloped virus like particles have a lipid layer [13]. Sometimes this layer composed of glycoproteins facilitates the entrance and recognition of virus like particles [13]. Although enveloped virus like particles are superior in terms of recognition than naked virus like particles, it requires more complexed system to produce [12]. Chimeric virus like particles are virus like particles made of more than two different viral components [14]. Filamentous chimeric virus like particles are featured with the high number of functional units [15]. Spherical chimeric virus like particles are featured with encapsulation of drug molecules [15]. Both molecules can undergo chemical conjugation or genetic modifications to attach foreign ligands [15]. A typical chemical conjugation needs a linker that binds to epitopes, with another end binding to the ligand [14]. Nevertheless, the most efficient method is with genetic engineering, where the chimeric virus like particles is engineered with desired ligand binding to epitope in genetic sequence [5].

Apart from the structure, virus based vaccines can also be classified in terms of their virus sources: plant virus, human virus, and animal viruses, where each field has made different extents of progress. In the following texts, represented vaccines from each source will be introduced.

3. Plant virus based vaccine

Plant viruses are featured with low immunity compared to animal viruses, since they cannot infect mammalian organisms. They can be engineered flexibly with chemical or genetic approach, since they have a few coat proteins to be modified, which ensures the effective introduction of various antigens [16]. Also, plant viruses in nature do not consist of replicating nucleic acids, which lowers the risks of reverse transcription. Finally, plant virus vectors can be produced from various cell lines such as bacteria, yeasts, plants, and animal cells [17]. They can be introduced into plants for ingestion. For instance, Alfalfa mosaic virus (AMV) vectors was introduced into spinach leaves, which makes these leaves express rabies epitopes. Ingesting these spinach leaves appears a profound increase levels of antibodies against rabies virus in human bodies [17]. Apart from edible vaccines, plant virus vaccines have taken an active part into treat different diseases.

Plant virus vaccines have been designed to fight against infectious diseases. For example, plant virus vaccine against malaria was designed with thrombospondin-related adhesive protein (TRAP) chemically conjugated to Cucumber mosaic virus (CMV) VLPs [18]. Also, plant virus vaccines have touched veterinary medication field, such as using canine parvovirus based vaccine to protect dogs from Cowpea mosaic virus (CPMV), using Bamboo mosaic virus to protect swine from foot and mouse disease virus (FMDV), and using CMV-based vaccine to protect pigs from porcine circovirus (PCV) [18].

Plant virus vaccines also have been designed to fight against cancer. Most anticancer vaccines are subject to low efficacy, compared to radiation and chemotherapy. However, virus vaccines can be good candidates since it has multiple epitopes that can induce profound T cell responses [9]. Also, its optimal size can enter lymphatic node and be uptake by APC [9]. In terms of breast cancer with HER2 receptor, plant virus vaccines show evident immune responses compared to other virus vaccines. The plant VLPs from CPMV shows a better therapeutic effects, including high number of IFN γ , IgG2a antibodies, and activation of CD4 $^{+}$ /CD8 $^{+}$ T-cells [18].

Finally, plant virus vaccines are involved in the treatment of allergies and autoimmune diseases. To prevent Alzheimer, plant virus vaccine have been designed as CMV carrying T-cell epitopes and conjugated to Abeta1-6 peptide. Mice injected with the particles reveal a noticeable Alzheimer plaques, which shows the induction of antibodies against the disease [19]. To reduce peanut allergy, an allergy which causes the highest number of deaths among food allergies, CMV VLPs carrying allergens Arah1 and Arah2 have been designed [18]. To limit cat allergy, which influences around 10% of the human population, CMV VLPs are genetically engineered with Fel d1 allergen, which profoundly stimulates the production of Fel d1-specific IgG antibodies and protects immunized mice from cat allergy [20].

In conclusion, plant based virus like particle vaccines have been involved in multiple fields. Although none of these vaccines are been licensed, most of them are under clinical trials and have shown remarkable results.

4. Human Virus based vaccine

There are plenty of virus like particle vaccines made from human viruses, these viruses include human papillomaviruses (HPV), hepatitis E (HEV), and hepatitis B virus (HBV). HBV virus like particle vaccine is the most approved vaccine globally, which are Heberbiovac HB, Gen VacB, GenHEvac B, Hepavax-Gene, Euvax B, recombivax HB [21,22]. Many of these vaccines are based on cloned surface epitopes. They enhance the antibody levels in most of individuals and provide long lasting protection for the human body, which on average protects for 20 years [23]. Vaccines like recombivax HBV appear strong protection against multiple HBV genotypes A and C [24]. More research needs to be done in this field since there are still a large number of mutants reported that is not protected by the vaccine mentioned above. Also, it is important to note that the time point of receiving vaccination is important. This paper notes that 36-year old immunized volunteer produces much less antibody against HBV than the 32-year old counterpart [23].

People are protected from HPV by two virus like particle vaccines Cervarix and Gardasil vaccines, where Gardasil vaccines have two versions, Gardasil-4 and Gardasil-9 [25]. All of these vaccines combined provide 80% of cervical cancer cases, and protect people from nine genotypes which are HPV 6,11,16,18,31,33,45,52,58. Among these genotypes, HPV 6 and 11 are the major cause of genital warts, which can be associated with 90% of cases [26].

All of these vaccines follow a similar design principle, deriving molecules from coat protein L1 to serve as antigens in human bodies [26]. Different HPV vaccines provide protection against different genotypes: while Cervarix provides cross- protection against HPV 35,31,33,45, Gardasil-4 provide antibodies against HPV 6,11,16, and 18 [27]. Therefore, it is important to note that individuals need to receive two to three HPV vaccines to receive a broad protection from HPV. The longevity of protection is also secured, where Cervarix and Gardasil-4 provide long term protection ranging from

11 to 14 years. Other potential HPV vaccines are in clinical trials which protect against more HPV genotypes. 14-valent candidate vaccine protect people from HPV genotypes that are not covered by current vaccines, including HPV 35,39,51,56,59 [28].

Apart from the licensed virus like particle vaccines, there are also many virus like particles under construction and testing. Multiple expression systems and source of structural proteins were tested. Structural proteins VP1 and Vp2 in vaccines against parvovirus B19 are produced from two different systems, which are *S.cerevisiae* and Sf9 insect cells [29]. While the former antigens induce a high antibody rate and persist for more than 80 days in immunized mice, later antigens reported 73% of reactogenicity in human participants [29]. More animal and human research needs to be done on parvovirus B19 vaccine to compare the effectiveness of vaccines on organisms. Other virus like particle vaccines use different structural protein sources. For instance, two vaccines against human immunodeficiency virus type 1 (HIV 1) derive either envelop or protein coat for antigens [30]. Many of these testing vaccines show promising effects on immunization. A testing vaccine against zika virus protected immunized AG129 mice from 0 death rate after 21 days [31].

All of the licensed vaccines are human virus like particle vaccines and provides broad protection for people against multiple variants of viruses. While some of the escaped virus variant exists, vaccines with broader protections are under clinical trials and will be licensed in the future.

5. Animal viruse based vaccine

Other animal virus vaccines are mainly derived from pigs, chickens, rabbits, horses, and fish [paper]. Most of the sources of virus vaccines are capsids, like vaccines against PCV-2, Porcine parvovirus(PPV0), and bluetongue virus [32]. One exception is a vaccine against Canine influenza virus(CIV) H3N2 variant, using envelope protein as its source [33]. Most of these animal virus like particle vaccines have shown superior results in animal testing than the licensed vaccines. For example, virus like particles derived from PCv-2 showed much higher antibody levels than Ingelvac CircoFLEX in mice, which is a commercial vaccine [32]; animal virus like particles vaccines from Influenza A virus showed a 42% higher immunization rate than commercial inactivated H6N2 vaccine [34]; vaccines from RGNNV showed 6.7% less mortality rate of immunized fish compared to the commercial inactivated vaccine [35].

Virus like particles from animal viruses also have been used as a vector for other virus prevention. Virus like particles against the influenza virus are derived from infectious bursal disease virus to mimic HA and M2 protein epitopes, keeping all of the immunized mice from 83% death rate [36]. Also, one of the foot-and-mouth disease virus vaccines is derived from the rabbit hemorrhagic disease virus to produce a T-helper epitope. The vaccine induces antibody IgA and IgG in pigs with high IFN-gamma-secreting cells [11].

Unlike human virus like particle vaccines, most of animal virus like particles are being used to produce vaccines that induces higher levels of immune response than the licensed vaccines. Other particles are utilized as a vector to treat viruses different sources.

6. Discussion

Virus like particles provide a promising future in vaccine fields since it has high ability to induce immune response with multiple T-helper cell epitopes to induce immune response; it has optimal size and shape to get into lymphatic system to getting captured by APC; it can also be engineered with encapsulate endogenous adjuvants to stimulate immune response [10, 37].

Current popular recombinant vaccines are mRNA vaccines. Compared to the mRNA vaccines, virus like particles vaccine do not need freezing conditions during transportation and storage [11]. Other advantages it that virus like particles can quickly induce immune response after being injected into human bodies, where the particles can immediately induce immune response after being

recognized antibodies. In contrast, mRNA vaccine needs to undergo transcription and translation to produce desired proteins.

Other popular vaccines are inactivated vaccines. Virus like particle vaccines can also be a good candidate to supplant them. Inactivated vaccines are viruses which being treated with hyper-temperature or inactivation chemicals. During the inactivation process, the protein structures of viruses may be modified and therefore, has the risks of leading to compromised immunogenicity [38]. Compared to inactivated vaccines, virus like particles just mimic the structure of viruses instead of modifying the structures. Therefore, Virus like particle is a much safer alternatives to inactivated vaccines.

Also, viruses like particles have multiple epitopes at the surface, which renders the benefits that they can induce Immunol response at a lower dose as 0.3 micrograms [39]. However, a novel study has revealed that the smaller doses may lead to less cross-protection, especially to those viruses with a lot of genotypes [40]. For example, HBV has over 10 genotypes [26]. It was suggested in the study that at least 10 micrograms of virus like particles in Engerix-B vaccine need to be injected to enhance cross protection from viruses with multiple genotypes [26,40]. Therefore, it is still important to retain high concentrations of virus like particles and inject vaccines with multiple trials to ensure that the immunization is formed, and cross-protection is secured. Moreover, there are still many virus variants that is not covered by the virus particle vaccines. HPV has more than 20 variants [26], whereas current vaccines only cover 9 of them. There are still some HPV types like HPV 35,39,51,59 associated with 10% of cervical cancers [26]. It is important to promote the studies of virus like particles vaccine to cross protect people from all variants of viruses.

7. Conclusion

This literature provides an overview of virus like particles vaccine in a different view from the conventional paper, where it was usually classified based on structures. In conclusion, virus like particle vaccines can be classified into three classes: plant virus like particles, human virus like particles, and animal virus like animals. Most of the commercial virus like particles are human virus like particles such as commercial HPV vaccines: Gardasil-4, Gardasil-9 and Cervarix. These vaccines provide cross protection among different genotypes of viruses. Among these virus-like particles, plant virus like particles vaccine takes an active part in multiple fields such as cancer, allergy, and autoimmune disease. Animal virus like particles are great candidates to replace current virus vaccines in the market, since most of them have better immune responses in clinical trials. However, more studies need to be done on human bodies to confirm the security and effectiveness of animal virus vaccines.

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