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Overview on Virus Like Particles from Plants Used as Vaccine Antigen

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ABSTRACT

Introduction: Virus-like particles (VLP) extracted from medicinal plant products are used and applied for the development of recombinant vector vaccines against various infectious diseases. This vaccine is mainly used for stimulating protective and long-lasting immune responses. In general, these vaccines mainly target the antigen-presenting cells (APC) or dendritic cells which may induce both humoral and cellular immune response. VLP based vaccines have been used so far in drug delivery, genetic therapy, cellular targeting, cancer treatment, therapeutic vaccine, etc.

Aim and Objective: This paper mainly reviews the existing developmental efforts through researchers to improve the production of plant virus particles based vaccines.

Results: However, the antigenicity and immunogenicity of such vaccines have various limitations varying from the length of the peptide that can be expressed on the surface to the duration of immunogenicity developed in the host. In other words, plant virus-like particles may provide some many benefits to the vaccine industry but still showed some challenges that limit the production of vaccines. Various efforts were taken or still ongoing about producing an efficient vaccine for human and veterinary related diseases.

Conclusion: Recently, plant virus-like particles are used for the development of vaccine platforms and have been tested in human and veterinary studies, suggesting that plant virus-based vaccines will be introduced into clinical and veterinary practice shortly.

Key Words: Virus-like particles, Plant, Immunity, Vaccine

INTRODUCTION

The concept of the vaccine developed by Edward Jenner against infectious diseases in the late Eighteenth century has seen a major development in the last few decades. The vaccine development industry has seen a shift from the use of whole organism (attenuated and inactivated) vaccines for the irradiation of Measles, Mumps, Rubella, Influenza etc., to the development of purified antigen vaccines/DNA vaccines/recombinant vector vaccines for disease such as hepatitis B[1,2]. This shift was witnessed as a result of the shortcomings of attenuated and inactivated vaccines. Where one presented the fear of less stable vaccine that may revert to its virulent form(attenuated vaccine), the use of other gave a less satisfactory immunogenic effect that required multiple boosters (inactivated vaccine). These lead researchers to look for an alternative method of vaccine development[3,4]. The new pathway that paved the way for stable vaccine development came with the understanding that immune response can also be stimulated by certain immune stimulatory factors (such as inactivated exotoxins, capsular polysaccharide, genetic material etc.) of the pathogen instead of using the entire pathogen itself[5,6].

One of such new vaccine developed using parts of the pathogen was the subunit vaccines based on VLP[7]. The first subunit vaccine was developed using yeast cell and antigen from hepatitis B virus. VLP is the virus particle without the genetic material having the same spatial conformation as the actual virus. VLP based vaccines present a major biosecurity property as it cannot replicate or revert into its virulent form given the absence of virulent genetic material[7, 8]. VLP can be

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used as a carrier platform for presenting the self-antigen to the host immune system or to develop a chimeric VLP that present nonself-antigen attached to the virus scaffold. The methods used for VLP modification include the chemical conjugation and fusion expression. Many VLP based vaccines have been licensed and commercialised since the late 1980s and many are undergoing clinical trials as of now[7-10]. Some of the viruses used so far include Rotavirus, H1N1, H5N1, Hantavirus, Dengue virus, etc. However, a new approach for vaccine development also demands an inexpensive method for their production[1, 2]. Plant as a bioreactor for the production of vaccines and pharmaceutical products have long been accepted as a cheap and easy to upscale method. This has involved the development of a vaccine for influenza and HIV. The modern pharmaceutical industry is still to tap into the huge plant-based resources as well as their use as a bioreactor for production of medicines[11, 12]. However ethical issues and controversial public perception of transgenic plants have led to the use of plant virus instead of the plant itself for production of antigen protein under a short duration of time. The plant virus such as *Cowpea mosaic virus* (CPMV); *Hibiscus chlorotic ringspot* virus (HCV); *Potato virus X* (PVX); etc., as scaffolds for encapsulating the antigen has presented as a strong immunogenic factor with no biosecurity issues[13].

IMMUNOSTIMULATORY PROPERTIES OF PLANT VIRUS BASED VACCINE

Interaction of VLP based vaccines follows the same rules as a typical virus particle. VLP is the perfect antigen-presenting platforms with high immunostimulatory properties. They are capable of inducing both humoral and cellular immune response and capable of displaying the heterologous antigen. Their particulate size, highly repetitive structure and ability to activate the immune response which mainly contributes to successful vaccine development[7-10].The highly repetitive structure on the plant virus particulate can be recognized by toll-like receptors (TLR) and taken up by the APCs having MHC class I. The multimeric viral pattern is highly efficient in cross-linking B cell receptors (BCR) for the activation of naïve B cells. These are then used for the activation of CD8 T cells. Other pathway includes the interaction of virus particles with dendritic cells, which then stimulates the innate immune system[14, 15].

To understand the immune response against tumour antigen when attached to virus-like particles i.e. potato virus X (PVX). These studies mainly suggest that idiotypic (Id) - IgG derived from BCL1 B lymphoma cells was cross-linked with PVX plant viral particle via biotin/streptavidin (Id-PVP) and showed higher anti-Id antibody response (sevenfold) in mice model as compared to weak immunogen Id was administered[16, 17]. In this study, researchers also investigated the interaction of Id-PVP with mice TLR.TLR7 is the only mice TLR to recognise ssRNA and activate the immune system. TLR7 plays an important role in the fight against the mammalian virus by inducing the secretion of IFN-α[18]. In other words, these studies claimed by these researchers which showed the production of IFN-α in the mice spleens through the interaction of PVP ssRNA or coat protein with TLR7. Hence PVX proved to be a safe carrier for inducing antibodies in human against Id antigen with no underlying immunity issue against them[16, 17].

Apart from the interaction of VLP with TLR and other immune factors for the production of antibodies, such induced immunity has been observed to be inefficient against chronic viral infection. Many times the administration of VLP also requires the corresponding adjuvant to obtain satisfactory antibody count in the host system. Along with the elimination of the infectious agent, it is also important to eliminate the infected cells using CTL response through cellular immunity[16,17,18]. For this, *Papaya mosaic virus* (PapMV) linked with p33 immunodominant CTL based epitope (acquire through LCMV) were administered into mice and their interaction with various antigen-presenting cells (APCs) were studied. This study was conducted to understand the immunization using PapMV-p33 against LCMV[19]. Dendritic cells (DCs) showed the maximum interaction with labelled PapMV-p33 among all the APCs. The PapMV VLPs were efficiently internalized, cut, processed and presented to naïve T cells. Vaccinated mice with PapMV-p33 were then challenged with LCMV to evaluate CTLs response[19].

PLANT VIRUSES BASED VACCINE DEVELOPMENT

Plant viruses (**Fig.1**) have been studied extensively to use them as an expression vector in the pharmaceutical industry. TMV along with CPMV may be one of the most studied carrier systems. Though they have successfully converted into nanoparticle vaccines however what both of them do not have in common is their structure[20,21]. CPMV possess an icosahedral structure that can be used for short repetitive epitope presentation on the capsid surface. Another approach involves an empty particulate structure that can be used for encapsulating drug or imaging factors for tumour detection[20].

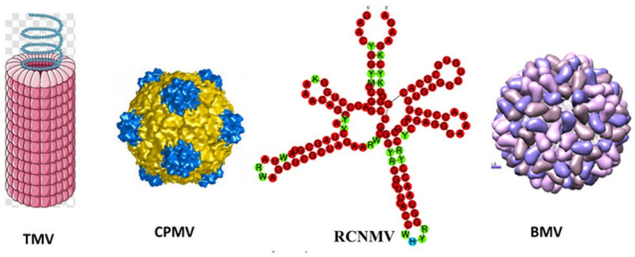

Figure 1: Structure of plant viruses.

CPMV because of their high yield, stable structure and lack of infectivity in mammalian cells became the perfect platform

for the presentation of epitopes for vaccine formation. Multiple short peptides (epitopes) are attached to a self-assembling CPMV vector that induces the production of antibody into the host cell. CPMV vector was conjugated with epitope from *Human rhinovirus* 14 (HRV-14) and was able to act as an immunogen for the same when administered into rabbits[22]. CPMV vector can be used for not only inducing antibody production in the host body but could also be used as a protein expression system for the production of full antibodies against infectious disease[20, 22]. Other plant viruses with the icosahedral structure are *Red clover necrotic mosaic virus* (RCNMV), *Brome mosaic virus* (BMV), *Hibiscus chlorotic ringspot virus* etc.

However, TMV possesses a helical rod structure that can function as a biocatalyst and an efficient drug delivery system. TMV is used for drug delivery system using conjugation and encapsulation method. Chemotherapeutic doxorubicin drug encapsulated into TMV particles were tested for efficient drug delivery, cell targeting and cell killing[21]. The test was conducted on two different cell lines i.e. (MDA-MB-231 and MCF-7; breast cancer) and successfully developed bioconjugation and encapsulation techniques for loading of therapeutic cargos in virus particles. Fluorescence and biotin labelling helped in conjugated protein and peptide structure evaluation and fluorophore helped in quantitative studies. Similar studies done on Cowpea mosaic virus shows the drug loading capacity of TMV rods is comparable to the 30nm icosahedral structure of CPMV[20,21,22].

Another study with serum albumin (SA) coated TMV showed enhanced tumour targeting property of the virus nanoparticles. The SA coated TMV accumulate in the tumour vasculature and tumour interstitium indicating towards efficient imaging as well as drug delivery system[20]. TMV rod shape particles can hence be loaded with either therapeutic drug Doxorubicin or imaging agents. Similar studies performed in Potato virus X (PVX) shows that the plant viral derived drug system is more efficient than free drug delivery system[23]. Though the number of *in vivo* testing involving plant VLP have been limited, the results of the already conducted experiment show a potentially improved system for drug delivery as well as imaging and detection of tumour cells. Other plant viruses with helical rod structure are *Potato virus* X (PVX), *Papaya mosaic virus*(PapMV), *Zucchini yellow mosaic virus*etc.[23, 24]

EXAMPLES OF PLANT VIRUS PARTICLES ARE CURRENTLY UNDER INVESTIGATION

Bamboo mosaic virus (BaMV; *Potexvirus* genus; family *Alphaflexiviridae*; **Fig.2**), contains a positive-sense single-stranded RNA, non-enveloped virus (width 15 nm and modal length 490 nm) and mainly infected both mono- and dicotyledonous plants. As per the literature, helical BaMV is considered a viral vaccine delivery system which may carry some larger transgene loads and showed higher immunity against various infectious diseases in animals with fewer side effects. Also, several pieces of evidence were reported and proved that dipterans as one of the major vector of BaMV. But it majors function is still unknown about BaMV which may circulate within the flies hemolymph and reached into the salivary gland to be expelled or whether BaMV should replicate within the dipterans[25]

Figure 2: BaMV.

Figure 3: CdMV.

Cardamom mosaic virus (CdMV, family Potyviridae; genus *Macluravirus*; **Fig.3**), appears asflexuous filamentous particles (650 nm length and 10-12 nm diameter). This disease is widely widespread and there is no effective means about control the virus transmission. One of the most common observations is seen in the morphology of diseased plants where interrupted pale green colour stripes which are mainly

reported on the veins and run parallel to each other i.e. started from midrib region to leaf margin. In advanced stages, pale green stripes are widely distributed on the leaf surface which gives a distinct type of mosaic pattern. This CdMV is transmitted through the banana aphid, *Pentalonia nigronervosa* and mainly infected major part of the rhizomes. In general, CdMV exists as a symptomless carrier or showed some mild symptoms which are reported in the planting material. One of the studies claimed that coat protein of CdMV), assembles into VLPwhen expressed in an *Escherichia coli* expression system. N and C-terminal of the coat protein were engineered with the Kennedy peptide and the 2F5 and 4E10 epitopes of gp41 of HIV. In some cases, chimeric type of proteins generally reacted with sera of HIV positive persons and also stimulated cytokine secretion from peripheral blood mononuclear cells. Overall, the coat protein of CdMV is mainly used about display HIV-1 antigens[26, 27].

Johnsongrass mosaic virus (JGMV, family *potyviridae;* **Fig. 4**), flexuous filamentous virion (modal length of 773–778 nm)firstly reported in *Sorghum halepense* (Johnsongrass) and Zea mays from Australia. This disease is mainly transmitted through aphids but showed some potential advantages. In literature, JGMV coat protein-containing N- and C-terminal regions which may not be required for its auto assembly but presented B and T-cell based epitopes simultaneously. In contrast, genetically engineered, JGMV RNA, including the gene of coat protein fused with the desired type of foreign epitopes to produce VLP in plants. This type of plant material is more effective in terms of oral delivery vaccine[28].

Figure 4: JGMV.

Figure 5: PapMV.

PapMV (family *Flexiviridae*; genus *Potexvirus*), single-stranded RNA mainly surrounded by a capsid which is made up of single viral encoded protein. In literature, novel nanoparticle adjuvant is reported which is mainly comprised of PapMV CP assembled with RNA. Overall, PapMV vaccine platform, mainly facilitate the designing of efficient vaccines and hopefully showed some effective response against various infectious threats[29]. Similarly, *Papaya ringspot virus* (PRSV; family *Potyviridae*; genus *Potyvirus*), pathogenic plant virus (non-enveloped, flexuous rod-shaped particle) which primarily infects the papaya tree. Plum pox potyvirus (PPV; family *Potyviridae*; genus *Potyvirus*), reported virions (764 X 20 nm) and ssRNA (9.7 kb) and normally infected various fruit species e.g. apricots, plums etc.

CONCLUSION

In short, VLPs are utilized in the form of nanoparticles and these are more safer and economical tools for prophylactic and therapeutic purposes e.g. cancer and other immune disorders. So, screening of plant virus-based nanoparticles or VLPs or VNPs are tried to use as one of the platform for screening of multiple copies of various immunogenic antigens exist on various pathogenic micro-organisms. In other words, these may be considered as suitable antigen carriers in case of vaccine production and targeted both humoral and cellular mediated immune responses.

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