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Review Article

A review on Effectivity of Plant based vaccines in the treatment of viral diseases

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Abstract

Plant engineering technology has been working effectively since last 30 years. Commercialization of different product using plant engineering is encouraging us to develop effective treatment and this progress takes too much effort and time, but still many candidate vaccines for use in humans are in clinical trials. Virus-like particles (VLPs) are basically self-constructed structures departed from viral antigens which copy the organization of similar viruses but without viral genome. This technology offers several pros in terms of safety, immunogenicity and stability in production over vaccines derived from pathogen formulation. Now, many pharmaceutical companies are working in this technology to develop effective treatment against various diseases. This review discusses how plant engineering technology works for diseases and regulations relevant to the development of plant-based vaccines in the treatment of viruses like Hepatitis B, Ebola, Papilloma, Norwalk, Influenza, HIV and Covid-19.

Keywords: Plant engineering technology, Virus-like Particles, Pathogens, Antibodies.

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INTRODUCTION

Virus-like particles (VLPs) are basically self-constructed structures departed from viral antigens which copy the organization of similar viruses but without viral genome. They offer several merits in terms of safety, immunogenicity and stability in production over vaccines derived from pathogen formulation or subunit antigens and hence, have earned huge momentum as a premier vaccine platform.[1] While attenuated or killed pathogens promote powerful immune responses and are still the key source of protection from several infectious diseases, significant reversion of attenuated pathogens or restricted inactivation of killed pathogens in the vaccine has remained a considerable safety concern. Furthermore, for a great number of pathogens either a safe attenuated strain is not yet obtained or no tissue culture system is available to permit its sufficient propagation and production. The development of subunit vaccines via genetic engineering has efficiency to overtake whole pathogen vaccines and associated risks. Notably, vaccines made up of individual proteins hardly possess antigenic determinant sites in their native conformation and hence, are significantly less effective than whole pathogen preparations. As a result subunit vaccines often need larger and more frequent administrations of antigen along with adjuvants to stimulate the desirable immune responses. VLPs possess the best traits of whole-virus as well as subunit antigens for the development of vaccine. VLPs are deficit of viral nucleic acid and are noninfectious. Hence, are safer alternatives of vaccine than attenuated or inactivated viruses. Additionally, the potency of VLPs can be considerably increased over the native virus when immunosuppressive viral proteins are exempted from VLP composition. Also, any undesirable epitope modification by the inactivation of live virus can be eluded for VLP manufacturing that further confirms the VLP's immunogenicity. Given that VLPs act as an infectious virus structurally, they can generate humoral immune and effective cellular responses even without adjuvants, which are more effective than other recombinant antigens. In addition to this, VLPs are highly stable than subunit vaccines and that can be formulated with help of recombinant technology in expression systems with no requirement of the capability to aid viral replication [1-3].

Plants as Production System for VLPs

Plants offer a great substitute for VLP vaccine manufacture due to their ability of producing huge quantities of recombinant protein at cheaper costs. Their eukaryotic processing mechanism for the post- translational

modification and appropriate set-up of proteins, and the low-risk of introducing fortuitous human pathogens [4,5]. Plants do not need costly fermentation facilities for biomass production or the generation of duplicate facilities to scale-up production. Therefore, plant biomass generation and upstream processing capacity can be controlled and scaled-up in a desirable, capital-efficient way whichcannot be easily compared by recent fermentation-based technologies [6,7]. Certain VLPs were primarilyexpressed in plants and they gave appreciable results, however, these earlier trials had to suffer from several demerits including less VLP expression, plant-specific glycosylation of glycoproteins, and the lack of ability to produce VLPs with multiple proteins [8]. Despite the fact, these challenges have all been overcome by latest development of new plant expression systems and improvement in plant glycoengineering. For instance, the initial production of VLPs in plants was quite slow and it yielded very low product. This issue demonstrates the intrinsic boundaries of early expression systems based upon stable transgenic plants, involving the lack of powerful regulatory elements to operate adequate amounts of target protein accumulation and also undesirable position effects emerged by the uncertainty of transgene integration in plant genome [9,10]. Because of low production yield, VLP production became impractical and it considerably reduced the cost-saving merit of plants [11]. The challenges involved in VLP production speed and yield have been overcome by the development of plant virus-based transient plant expression systems [12,13]. The cloning and high-level transient expression of plant-derived VLPs is quite easy and can be acquired rapidly in 1–2 weeks of vector infiltration with a tobacco mosaic virus (TMV) RNA replicon (the MagnICON) system or a gemini viral DNA replicon system based upon bean yellow dwarf virus (BeYDV) [14-16]. These new technologies in the rate and quantity of yield of VLP production also offer the plant-expression system an important feature of high versatility in producing VLP vaccines in opposition to the viruses which mutate their surface antigens rapidly, hence fulfilling a specific advantage over other production systems in producing vaccines to cope with unavoidable pandemics (e.g., influenza A) in a timely manner. Likewise, the problem of plant-specific glycans has been satisfyingly overcome by the evolution of transgenic plant lines with “humanized” glycosylation pathways (see glycosylation section below). Additionally, effective production and fabrication of VLPs with up to three different kinds of proteins have also been obtained in plants [17,18].

Plant-Derived VLPs May Provide a Novel Vehicle for Delivery of Vaccines in the treatment of Diseases

Plant-derived hepatitis B surface antigen employed in oral immunization studies

Eliciting an immune response in humans through oral delivery of slightly processed plant material expressing vaccine antigens is achievable. Both vaccines tested till date was aimed opposing to enteric pathogens, namely, Norwalk virus [19] and entero toxigenic Escherichia coli [20]. Recently, this idea has been expanded to non-enteric pathogens with the satisfactory demonstration. Hepatitis B surface antigen (HBsAg), conveyed in transgenic potato tubers was immunogenic in animals when given orally [21, 22]. Approximately 2 billion people have been contaminated by the hepatitis B virus (HBV) and 15–17% of the infected characteristic as active carriers, with the maximum endemicity happening among developing countries [23]. A yeast-derived HBV subunit vaccine antigen has been successfully conveyed from plants. The HBsAg was found to gather intracellularly as tubules, with a complex size distribution, drastically varying from the VLP preparations of the current commercial vaccines. The present injectable vaccines possess the standard to compare. It has now been demonstrated that the plant-derived HBsAg was made up of long filaments packed within the Endoplasmic reticulum (ER) [24-29]. Also, the antigen was linked with the ER membrane itself, from which the filaments are derived [30]. This structure is fundamentally non-identical to the recent injectable vaccines, which comprise of VLPs with the uniform size of 20–22 nm [31, 32]. However, recently undertaken animal trials have revealed that the tuber-derived antigen was capable of eliciting a primary immune response signaling that a VLP structure was not critical for effectiveness of HBsAg as a vaccine. The extent of disulfide bonding would be more important, which is crucial for presentation of immunogenic epitope. Results have demonstrated that small HBV surface protein (SHBs) dimers predominate the plant-derived antigen acquired from all three recombinant systems. It has also been reported that the dimer form of SHBs possesses all the essential epitopes for immunogenicity [33]. The degree of intermolecular disulfide bonding spotted was identical to a yeast-derived vaccine which is currently being marketed [34]. Depending upon the expression system, from 21% to 37% of total SHBs were reactive to the Auszyme diagnostic kit, an immunoassay which is performed to determine the potency of any commercial vaccine [35].

In the treatment of Ebola virus

No counter measures available as of now for the management of the severe Filovirus (e.g., Ebola virus; EBOV) infection [36, 37]. Specifically designed monoclonal antibodies (mAbs) which could be used in humans as immune protectants for EBOV to overcome this limitation, starting with a murine mAb (13F6) which identifies the heavily glycosylated mucin-like structure of the virion-attached glycoprotein (GP). Point mutations were administered into the variable region of the murine mAb to discard predicted human T-cell epitopes, and the variable regions linked to human constant regions to produce a mAb (h-13F6) suitable for human administration. The potency of three variants of h-13F6 having diverse glycosylation patterns in a lethal mouse EBOV challenge model was evaluated [38, 39]. The flow of glycosylation of the various mAbs was established to compare to the degree of protection, with aglycosylated h-13F6 possessing the least potency (ED50 = 33 μg). A variant with typical heterogenous mammalian glycoforms (ED50 = 11μg) had similar efficacy to the native murine mAb. Although, h-13F6 possessing complex N-glycosylation lacking fundamental fucose provided enhanced potency (ED50 = 3μg). Binding studies with use of Fcγ receptors demonstrated increased attachment of non-gucosylated h-13F6 to human and mouse FcγRIII. Combinedly, the results confirm the existence of Fc N-glycans, which intensify the protective efficacy of h-13F6, and the mAbs produced with uniform glycosylation and greater potency of glycol form proposes a promising biodefense therapeutic [40].

In the treatment of Papillomavirus

Vaccines used for Human papillomavirus (HPV), Cervarix® and Gardasil®, consist of virus like particles (VLP) according to the primary capsid protein, L1, HPV16 and HPV18. Both vaccines are greatly efficient at preventing persistent infection and more progressive conditions related to HPV16 and HPV18 [41, 42]. Antibodies which are capable of containing pseudoviruses constituting HPV16 and HPV18 both can be observed in the serum and cervico-vaginal secretions of vaccines [43– 45]. Passive transfer studies establishing that

immune sera, purified IgG or monoclonal antibodies (MAbs) can defend animals from papillomavirus challenge [46–48], have led to the belief that neutralizing antibodies can help to achieve vaccine-induced type-specific protection [49,50]. The vaccines grant an extent of cross- protection against some genetically-related types from the alpha-9 (HPV16-like: HPV33, HPV31, HPV52, HPV35, HPV58) and alpha-7 (HPV18-like: HPV45, HPV39, HPV68, HPV59) groups of species. Cross-protection is concurrent with the observation of low titer serum responses against non-vaccine types by vaccines. Those kind of antibodies may be the mediator of detection and their cross protection may be helpful as a correlate or surrogate. Antibodies produced after Cervarix® were analyzed by pseudo virus neutralization after the vaccination on 13–14 year old girls, [51] VLP ELISA and by enrichment of target antigen specificity using VLP immobilized beads. Two-dimensional serology data recommended that, antibody specificity profile generated by VLP ELISA was qualitatively as well as quantitatively non- identical from the neutralizing antibodies specificity profile [52,53]. Target-specific antibody enrichment showed that cross-neutralization of non-vaccine types was because of minority of antibodies higher than by weak interactions of a predominantly type-restricted HPV16 antibody specificity. In addition to this, cross-neutralization of non-vaccine types appeared to be mediated by numerous antibody specificities, many non-vaccine types, recognizing single and whose specificities were not identifiable from examination of the serum neutralizing antibody profile. These data help to understand that the antibody specifications elicited after HPV vaccination and have remarkable implications for vaccine induced cross-protection.

In the treatment of Rabies virus:

Using engineered amino virus-based vectors is a novel approach to the manufacture and delivery of vaccine antigens. A chimeric peptide containing antigenic determinants from rabies virus glycoprotein (G protein) (amino acids 253–275) and nucleoprotein (N protein) (amino acids 404– 418) was PCR- amplified and cloned as a translational fusion product with the alfalfa mosaic virus (AlMV) coat protein (CP) [54-63]. This recombinant CP was indicated in two plant virus-based expression systems. The first one utilized transgenic *Nicotiana tabacum* cv [64] Samsun NN plants offering replicative functions in trans for full-length infectious RNA3 of AlMV (NF1-g24). The second one utilized Nicotiana benthamiana and spinach (Spinacia oleracea) plants using autonomously cloning tobacco mosaic virus (TMV) lacking fundamental CP (Av/A4-g24). Recombinant virus consisting of the chimeric rabies virus epitope was obtained from infected transgenic N. tabacum cv. Samsun NN plants and was utilized for parenteral immunization of mice. Mice immunized with recombinant virus were shielded against challenge infection. Depending upon the formerly demonstrated efficacy of this plant virus-based experimental rabies vaccine when administered orally into mice in virus-infected unprocessed raw spinach leaves, its efficacy in human volunteers was accessed. Three out of five volunteers who had formerly been immunized against rabies virus through a conventional vaccine specifically responded against the peptide antigen after consuming recombinant virus infected spinach leaves. When rabies virus non-immune volunteers were fed the same material, 5/9 demonstrated strong antibody responses to either rabies virus or AlMV. Three of the individuals showed detectable levels of rabies virus- neutralizing antibodies following a single dose of conventional rabies virus vaccine, whereas none of five controls produced these antibodies. This data provides a clear indication of the potency of the plant virus-based expression systems as supplementary oral booster for rabies vaccinations.

Table 1: Results of generation of antibodies in rabies

Antibody response and survival of C3H mice immunized with AlMV containing chimeric peptide from rabies virus. Group of mice	Codes	Dose of antigen (g per dose)	Rabies neutralizing antibodies	Survival afterChallenge with rabies virus
NF1-g24	A	250	4/5	5/5
	B		5/5	5/5
AlMV	C	250	0/5	2/5
	D		0/5	0/5
G5–24-N31D	E	25	1/5	1/3
	F		4/5	2/4

In the treatment of Norwalk virus

Recent studies using new diagnostic assays developed with recombinant NV (rNV) particles or using reverse transcription– polymerase chain reaction have shown that the epidemiologic significance of NV infections has been greatly underestimated [65–68].A new approach for delivering vaccine antigens is the use of inexpensive, plentiful, plant based oral vaccines [69,70]. Usage of inexpensive, plentiful, plant based oral vaccines is a novel approach for supplying vaccine antigens. Norwalk virus capsid protein (NVCP) structured into virus-like particles was utilized as a test antigen to find out whether immune responses could be produced in volunteers who consumed transgenic potatoes. Twenty-four healthy adult volunteers were given 2 or 3 doses of transgenic potato (n = 20) or 3 doses of wild-type potato (n = 4). Each dose comprised of 150 g of raw, peeled, diced potato that contained 215–751 mg of NVCP. 19 of 20 volunteers (95%) who ingested transgenic potatoes showed considerable increases in the numbers of specific IgA antibody–secreting cells. 4 of 20 volunteers (20%) developed specific serum IgG, and 6 (30%) generated specific stool IgA. Overall, 19 out of 20 volunteers developed immune responses of some kind, although the degree of serum antibody increase was modest.

Safety: No changes in the incidence rates of nausea,

vomiting, fever, mild cramps or diarrhea were observed among volunteers who ingested transgenic or wild-type potatoes within three days after the ingestion of the first dose of potatoes. The most common symptom observed was nausea, which occurred in 4 out of 20 (20%) of the volunteers who ingested transgenic potatoes and 1 of 4 (25%) volunteers who ingested wild-type potatoes. Cramps occurred in 5 of 20 (25%) volunteers who ingestedtransgenic potatoes and 2 of 4 (50%) volunteers who ingested wild-type potatoes [71].

Immunogenicity: 19 out of 20 (95%) individuals who ingested2 to 3 doses of transgenic potatoes showed significant raise in the numbers of IgA ASCs (range: 6–280/106 PBMC). The recipients of wild-type potato showed a geometric mean of 2 IgA ASCs/106 PBMC following 3 doses. 13 out of 19 IgA ASC responses were obtained following the first dose of transgenic potato. 6 out of 20 (30%) individuals showed significant raise in IgG ASCs (range: (25–115/106 PBMC)). These circulating cells which produce specific antibodies reflect immunologic priming of the immune system of gut mucosa [71].

In treating Influenza

Influenza virus infections cause a severe respiratory disease, because of which 3 to 5 million cases of serious illness are recorded worldwide yearly, including 250,000 to 500,000 fatalities. Since 2004, the virus has spread rapidly and now it has caused serious poultry disease outbreaks in many Asian countries along with Europe and Africa. Two main influenza surface antigens namely HA and NA have been expressed in-insect, bacteria yeast, plant and mammalian cells as soluble recombinant proteins. These were used successfully to induce protective immunity in animal models [72-77]. Recombinant soluble influenza proteins have already been tested in clinical studies for different age groups 9 [78-80]. More than 500 people have been contaminated with H5N1 virus with 50–60% mortality rate. Fortunately, human to human transmission hasn't been on higher side and occurred on rare occasion as most of the reportedhuman cases have had close contact with infected birds. Nevertheless, this doesn't minimize the concern for human health due to their severity of human cases and adaptive nature of virus which could mutate or re-assort and might develop the ability to spread effectively among humans. They are very effective and safe inducers potentially have broader [81] protective immune responses. In fact, InfluenzaVLP vaccines that are to be used for H1N1, H5N1, and H7N9 manufactured in different platforms have entered clinical trials [82-84]. Apart from VLPs, viral vectors carrying influenza antigens are also interesting Especially the recombinant modified vaccinia virus Ankara (MVA) vector has a very good safety profile in humans and preclinical studies with MVA-based pandemic influenza vaccines are of great potential [85,86]. An adenovirus vector-based H5N1 vaccine checked in a clinical observation in humans pretend that this type of vaccine may have a promising future for use with poorly immunogenicvaccines in a prime boost setting in which the adenovirus- based vaccination is followed by a parenteralbooster injection with inactivated vaccine [87,88].

In the treatment of HIV

The acquired immune deficiency syndrome (AIDS) is one of the most prominent diseases worldwide that is caused by the human immunodeficiency virus (HIV). Plant-based vaccines for HIV provide a topic of great interest to the researchers which are observed by great number of reports upon expressing HIV antigen in plants [89]. HIV is mostly transmitted via genitourinary and rectal mucosa where it enters by crossing the epithelial cells [90]. Developments on the manufacture and by putative protective proteins characterization at the antigenic level have showed the viability of this approach. Since mucosal immuneresponses could be efficiently induced by the administration of vaccines onto mucosal surfaces, which offers the possibility of leading to HIV immunity [91, 92]. Based on the current progress in this field, it is clear that a detailed immune logical characterization for a very large number of explored antigens is yet to be performed [93,94]. However, since eliciting specific and broad cellular and humoral responses are necessary requirement for prevention or reduction of severity of the HIV infection, it is a mandatory need to evaluate new protein configurations for identifying highly effective immunogens. Besides, some goals like co-expression of adjuvants will be viable by the trans plastomic technologies marking a hugecontribution in this field. These advances will help us a step ahead towards the next preclinical steps, which can lead us to neutralization of HIV. Ina nutshell, plant-based vaccines have open doors to an alternative, which along with traditional approaches, might be helpful in the fight against HIV [95].

In the treatment of Corona

Corona viruses (COVs) consist assorted group of positive-sense embedded RNA viruses having genomes which range between 27–32 kb [96]. A biopharmaceutical company from Canada named Medicago, has successfully made virus-like particles (VLPs) of the coronavirus around 20 days following the SARS-CoV-2 genetic sequence. Despite of opting for egg-based methods to produce vaccines, this technology an encoded genetic sequence of COVID 19 spike protein is inserted into Agrobacterium, a commonly found soil bacterium which then consumed by plants [97]. The resulting plants generate a particle identical to virus which constitute of plant lipid membrane and COVID-19 spike protein. Medicago is employing Nicotiana benthamiana, a plant that shares identical family to thatof tobacco plant, to form VLPs of SARS-CoV2 virus (COVID-19: Medicago's Development Programs). These VLPs are identical in shape and size to coronavirus but are devoid of nucleic acid and are thus noninfectious. After successful completion of Phase-1 clinical trials, Medicago is currently working on Phase-2 trials [98]. Medicago his experienced in developing VLPs comprised of influenza virus haemagglutinin before, and have reported their safety and efficacy in animal models and in human clinical trials, too [99]. The production of a plant- made vaccine based upon VLPs is quite cost effectivewhen compared to its conventional counterpart [100]. British- American Tobacco (BAT) through Kentucky Bio-processing (KBP), its biotech subsidiary present in US, is formulating an efficient vaccinefor COVID-19 and its pre-clinical trials are in progress[101]. Experts at KBP have cloned a piece of geneticsequence of SARS-CoV-2, which was utilized for potential antigen that was injected into the plants of *Nicotiana benthamiana* for production. The pre-clinical trials of vaccine have shown a positive immune response and will be into Phase-1 human clinical trials very soon [102]. BAT has a production capacity ofapproximately 1–3 million doses per week of COVID-19 vaccines (they prepared 10M flu vaccines in a month and Ebola vaccine utilizing the same plant based approach [103,104]

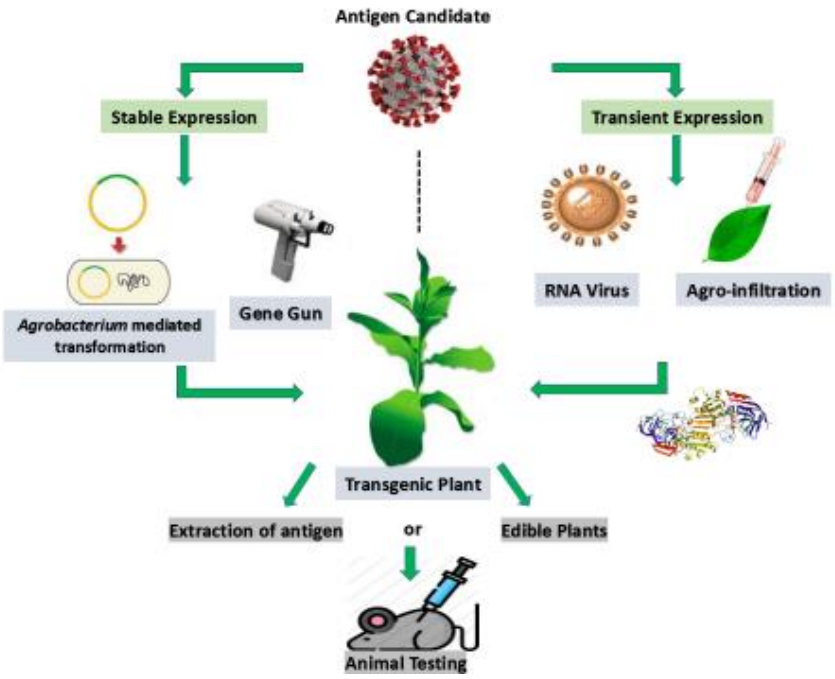

Figure 1: Schematic diagram of Plant pharming

CONCLUSION

The progress for commercialization of plant-based vaccines takes much effort and time, but it will definitely work in the future. They offer several merits in terms of safety, immunogenicity and stability in production over vaccines derived from pathogen formulation or subunit antigens and hence, has earned huge momentum as a premier vaccine platform. VLPs are deficit of viral nucleic acid and are noninfectious. Hence, are safer alternatives of vaccine than attenuated or inactivated viruses. Moreover, VLPs are more stable than subunit vaccines and can be manufactured with the help of recombinant technology in expression systems with no requirement of the capability to aid viral replication. This plant engineering technology works in the treatment of viruses like Hepatitis B, Ebola, Papilloma virus, Norwalk, Influenza, HIV and Corona. Many key points are essential for the development of a broadly effective GMP-compliant regulatory framework for clinical application of plant-based vaccines in humans. The challenge is to facilitate the procedures without compromising quality, which is a prerequisite for manufacturing plant-based human vaccines.

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Conflict of Interest: The authors declare that there is no conflict of interest

Abbriviations:

VLS: virus-like particles, **BeYDV:** bean yellow dwarf virus, **HBV:** Hepatitis B virus, **HBsAg:** Hepatitis B surface antigen, **ER:** Endoplasmic reticulum, **SHBs:** small HBV surface protein, **EBOV:** Ebola virus, **mAbs:** monoclonal antibodies, **ED50**: median effective dose, **HPV:** Human papillomavirus, **ELISA:** enzyme-linked immunosorbent assay, **PCR:** polymerase chain reaction, **AIMV:** alfalfa mosaic virus, **CP:** coat protein, **rNV:** recombinant NV , **NVCP:** Norwalk virus capsid protein, **PBMC:** peripheral blood mononuclear cell, **AIDS:** acquired immune deficiency syndrome, **HIV:** human immunodeficiency virus, **COVs:** Corona viruses, **KBP:** Kentucky Bio-processing, **BAT:** British- American Tobacco.

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