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Use of plant viruses and virus-like particles for the creation of novel vaccines

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ABSTRACT

In recent decades, the development of plant virology and genetic engineering techniques has resulted in the construction of plant virus-based vaccines for protection against different infectious agents, cancers and autoimmune diseases in both humans and animals. Interaction studies between plant viruses and mammalian organisms have suggested that plant viruses and virus-like particles (VLPs) are safe and noninfectious to humans and animals. Plant viruses with introduced antigens are powerful vaccine components due to their strongly organized, repetitive spatial structure; they can elicit strong immune responses similar to those observed with infectious mammalian viruses. The analysis of published data demonstrated that at least 73 experimental vaccines, including 61 prophylactic and 12 therapeutic vaccines, have been constructed using plant viruses as a carrier structure for presentation of different antigens. This information clearly demonstrates that noninfectious viruses are also applicable as vaccine carriers. Moreover, several plant viruses have been used for platform development, and corresponding vaccines are currently being tested in human and veterinary clinical trials. This review therefore discusses the main principles of plant VLP vaccine construction, emphasizing the physical, chemical, genetic and immunological aspects. Results of the latest studies suggest that several plant virus-based vaccines will join the list of approved human and animal vaccines in the near future.

Contents

1. Introduction

Vaccination is one of the most successful medical achievements of the XX century, preventing an estimated six million deaths per year [1]. From a historical perspective, vaccination as a public health intervention had already been appreciated for hundreds of years.

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The term "vaccination" was introduced by Jenner at the end of the XVIII century, when he successfully demonstrated the use of live attenuated material from infected cows (lat. "vacca") for protection of humans against smallpox [2].

The discovery by Luis Pasteur in 1881 can be considered a starting point of targeted vaccine development, as he hypothesized that elevated temperatures, oxidation and different chemicals can attenuate pathogens for subsequent use as vaccines. Later, vaccine development strategies, such as passages *in vitro*, cold adaptation, and auxotrophy, were introduced [3]. The significant idea about vaccines based on protein(s) originating from pathogens (subunit vaccines) came from the development of influenza, pertussis, anthrax and rabies vaccines [2]. It should be emphasized that the majority of current vaccines are still produced according to the above principles.

As in all other areas of biology and biotechnology, the development of gene engineering techniques has also significantly influenced vaccine creation. As a result, the cloning of viral structural genes and their expression in heterologous host cells have enabled virus-like particle (VLP) technology. In the early 1980s, this technology resulted in the creation of a new vaccine against hepatitis B (HBV) using recombinant yeast cells carrying the HBV S antigen coding sequence (HBsAg [4]). Recombinant yeast-derived vaccine development has allowed the large-scale production of noninfectious particles and has replaced the vaccine prepared from blood serum of chronically HBV-infected patients [5]. Additionally, electron microscopy visualizations confirmed the high similarity of *Escherichia coli*-produced VLPs with HBV core (HBcAg) shells that have been purified from human liver samples [6]. The observed structural equivalence between native viruses and VLPs is one of the most important principles of creating new nanomaterials, including VLP vaccines. Therefore, achievements in the production of recombinant viral structural proteins have been decisive for the initiation of the VLP era in vaccinology.

The efforts of researchers and technologists in subsequent decades have resulted in four types of prophylactic VLP-based vaccines out of the approximately 50 [7] licensed for human use, which are the following:

1) Vaccines against HBV: these differ in production systems and used antigens (Engerix and Recombivax – yeast system, HBsAg [4]; Sci-B-Vac – mammalian CHO cells, 3 antigens in the same VLP: 75–77% of HbsAg, 4–7% of preS1 and 17–21% of preS2 [8]);
2) Cervical cancer vaccine variants based on human papilloma virus (HPV) major structural protein L1 VLPs: vaccines differ in production systems and HPV L1 subtypes (Gardasil – yeast, 4 subtypes [9]; Gardasil-9 – yeast, 9 subtypes [10], Cervarix – insect cells/baculovirus, 2 subtypes [11]);
3) Hepatitis E vaccine, constructed from a single, truncated capsid protein of HEV and produced in *Escherichia coli* (*E. coli*) cells [12]: the vaccine is licensed for the Chinese market;
4) Malaria RTS, S vaccine, containing the fusion protein of the C-terminal part of circumsporozoite CS antigen and HBsAg, which is coexpressed in yeast cells together with unmodified HBsAg (20% antigen incorporation in VLPs; [13]). The newest version of the vaccine with enhanced efficacy represents a yeast-produced direct fusion between HBsAg and CS antigen and is being evaluated in human clinical trials [14].

In the future, after successful tests in different phases of clinical trials, an additional 30–40 prophylactic and therapeutic vaccines will join the list of approved VLP vaccines [15].

All of the abovementioned licensed vaccines are based on artificial VLPs derived from human pathogenic viruses, including the malaria RTS vaccine, where HBsAg serves as an antigen carrier. The carrier idea has been known since the 1980s from studies of peptide-derived vaccines, when protein carriers such as keyhole limpet hemocyanin, bovine serum albumin, diphtheria and tetanus toxoids [16,17] were used to stabilize antigenic peptides and enhance the immune response. One of the first VLP carriers to be exploited for peptide display in experimental vaccines was a plant virus - tobacco mosaic virus (TMV) [18]. The recombinant TMV VLPs presenting poliovirus epitopes on the VLP surface have turned out to be immunogenic and elicit the formation of antibodies against poliovirus in a rat model.

This study demonstrated several important principles for the construction of VLP-based vaccines. First, the construction process of VLPs is significantly facilitated if the data of basic research on the progenitor virus, including nucleotide sequences of structural protein genes, are available. Next, oligonucleotide-mediated synthesis of the coding sequence of viral coat and the chosen antigen considerably simplifies the gene cloning process by avoiding cDNA amplification from the native source. Further, viral coats tolerate antigen additions or insertions without affecting the characteristic viral structures; thus, cultivation of heterologous, recombinant hosts enables VLP production in nearly unlimited amounts. Finally, the study clearly demonstrated that, after corresponding modifications, nonpathogenic viruses can also be adapted for vaccine purposes.

In this review, we attempt to summarize the publicly available data on plant virus-like particles and vaccines derived from them, emphasizing aspects of vaccine construction. Two databases were used as a source of information: Web of Science (https://apps.webofknowledge.com) and PubMed database of the US National Institutes of Health (https://www.ncbi.nlm.nih.gov/pubmed/). In this paper, we use the term "viral particles" for native and recombinant infectious viruses and the term "VLPs" for noninfectious virus-derived structures generated from viral structural genes. The graphical summary of this review is presented in Fig. 1.

2. Plant viruses and VLPs: a short overview

Plant viruses are small plant-infecting agents that require host cells for their lifecycle. In nature, plant viruses are transferred from infected to uninfected plants by mechanical injury of plant tissues, mainly caused by insects feeding on plant leaves or stems. Thus, plant viruses use fundamentally different expansion strategies compared to those of mammalian or bacterial viruses, which infect susceptible organisms using receptor mechanisms.

Plant viruses have been intensively studied since the end of the XIX century, when Adolf Mayer discovered the agent that causes tobacco mosaic disease, and Beijerinck used the term "virus" for this agent. For more than 100 years, tobacco-infecting TMV has served as a model virus for research and has significantly contributed to our understanding of viral structures, assembly mechanisms, and viral lifecycle, as well as biotechnological applications of viruses, including model vaccines [19–21]. Along with viruses, such as tomato spotted wilt virus (TSWV), tomato yellow leaf curl virus (TYLCV), cucumber mosaic virus (CMV) and potato virus Y (PVY), TMV remains the most important virus based on evaluations of scientific and economic significance [22].

According to the IX Virus Taxonomy report of the ICTV, there are approximately 900 species of plant viruses [23]. This number mainly represents viruses that are isolated from agricultural crops and can only be a minor part of naturally existing species.

Plant virus genomes are built from nucleic acids (DNA or RNA), which encode at least three types of viral proteins: polymerases for viral genome replication, movement proteins (MP) necessary for virus spread from one plant cell to another, and hundreds or thousands of coat protein (CP) molecules protecting the viral genome from environmental stress. Plant viruses utilize different strategies for gene expression of encoded proteins, such as polyproteins, translation from subgenomic RNAs, ribosomal frameshift signals, internal ribosome entry sites and "leaky-stop" codons to achieve maximal coding capacity of the genome. Knowledge of these strategies is highly important for the construction of virus-based plant expression systems (see Chapter 3.4). These and other aspects of plant viruses are discussed in Refs. [24–26].

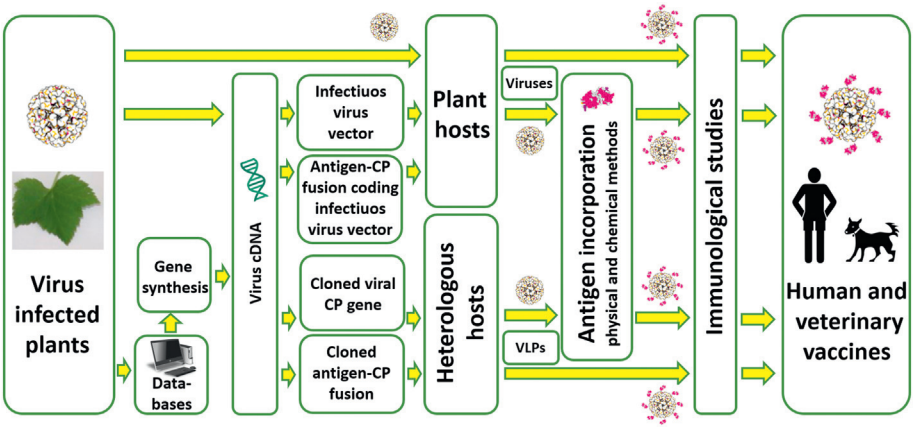

Fig. 1. Overview of typical steps in construction process of plant virus-derived vaccines.

One of the most important milestones in the development of plant virus-based molecular technologies is the discovery made by Gierer and Schramm in 1956, who demonstrated that RNA of native TMV alone can be infectious [27]. Further development of recombinant technologies has resulted in the complete genomic sequence of TMV [28] and has allowed targeted manipulations with viral nucleotide sequences, which resulted in the first artificially synthesized, infectious cDNA of a plant virus in 1984 [29].

Structurally, a significant part of plant virus particles is organized into flexible or rigid rod-shaped structures, while other viruses are isometric with icosahedral symmetry. Examples of some plant virus structures used in the construction of vaccines are shown in Fig. 2. Achievements in three-dimensional (3D) structural studies of viruses are the next turning point in technology development, which allow the prediction of exposed regions in viral structure for the surface presentation of antigens and significantly facilitate the construction of efficient recombinant VLP vaccines. The very first high-resolution icosahedral virus structural model has been suggested by Harrisson and coworkers [30] and is based on tomato bushy stunt virus (TBSV) crystallographic studies. TMV is the first helical virus for which the 3D structure has been determined using fiber diffraction [31]. Additionally, this study clearly demonstrated the spatial position of viral nucleic acids in the viral particle. Based on these pioneering studies, numerous plant virus structures have been elucidated using crystallography and fiber diffraction methods. Additionally, the latest progress in cryoelectron microscopy (cryo-EM) has allowed determination of the near-atomic structures of flexous helical viruses, such as potexviruses [32]. In some cases, the CP subunits of flexible viruses are crystallized and their atomic structures resolved; the crystal structure and cryo-EM data combination can be used to build a virus spatial model [33].

As already mentioned, the first plant VLPs were constructed from TMV and were obtained after structural gene expression in bacterial cells. Since then, plant viruses have been widely used for different nanotechnological applications, including vaccine carriers. In the literature, we found 55 different species of plant viruses belonging to 29 virus genera that have been shown to be useful for these purposes, taking into account native and different variants of recombinant infectious viruses and their noninfectious VLP analogs (see Supplement Table 3; the data

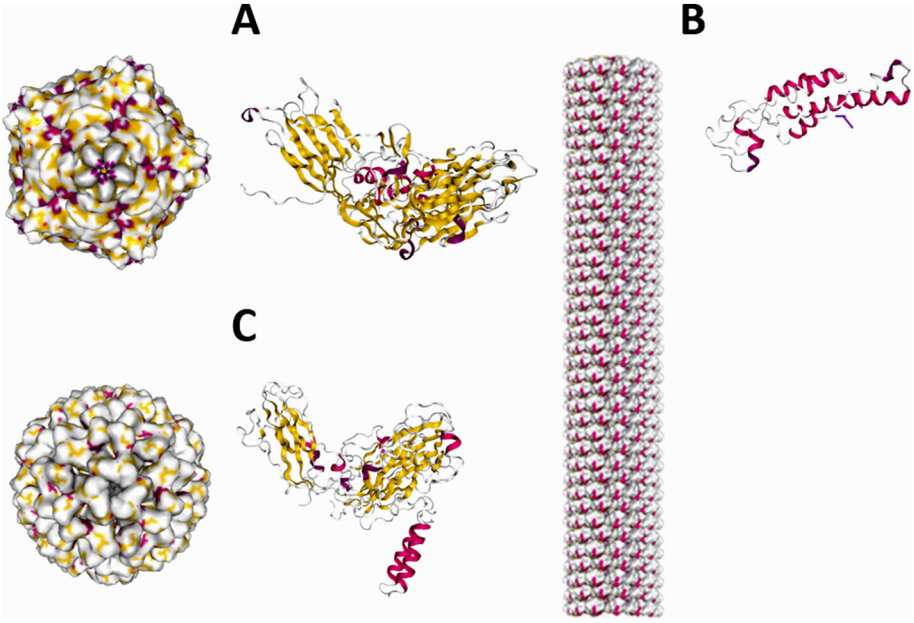

Fig. 2. Structure examples of icosahedral and helical plant viruses, used for vaccine development. Images were created using Protein Data Bank and NGL 3D viewer [147]. The surface model of the virus is shown on the left side of corresponding panel, on the right – side view of the coat protein(s) structure. α-helices are shown in red, β-sheets – in yellow. A – Cowpea mosaic virus (CPMV) structure (T = 3 symmetry, diameter - 28 nm). Image of 1NY7 [148]. B – Tobacco mosaic virus (TMV) structure (cryo-EM reconstruction of a TMV fragment; particle length – 300 nm, diameter - 18 nm). Image of 3 J06 [149]. C – Cucumber mosaic virus (CMV) structure (T = 3 symmetry, diameter - 28 nm). Image of 5OV6 [65].

are grouped according to virus genera). Significant parts of the viruses (21 virus species) have been tested as antigen carriers for different vaccines, including 8 isometric and 13 helical virus derivatives.

The fact that plant viruses are able to elicit antibody formation in mammalian organisms has been known since 1940s [34]; later, that idea resulted in commercial use of plant virus-specific antibodies as sensitive tools to identify infected plants in agriculture. In previous decades, immunologists substantially changed our basic knowledge about viral immunology [3], resulting in impressive achievements in viral and VLP vaccine development, by far exceeding the simple generation of antibodies for identification of virus-infected plants. Several excellent review articles about nanoparticle and VLP immunological properties, including plant virus-derived vaccines, have recently been published [35–41].

3. Construction of vaccines

One of the most important properties of VLPs is the ability to mimic the macromolecular structures of viruses and induce in mammalian organisms strong immune responses that are comparable to those induced by native, infectious viruses [35]. The immune system recognizes the viral and virus-like particles based on the following characteristics [42], which have to be considered in the vaccine construction process:

1) Viral and VLP surfaces are highly structurally organized and contain repetitive amino-acid (AA) stretches built up of several hundred to thousands of viral CP molecules; these repetitive structures can crosslink B cell receptors, leading to strong stimulation of B cells and inducing long-lasting antibody responses. From the vaccine construction point of view, viral particles and VLPs have to retain their typical viral morphology after all manipulations;
2) The size, shape and rigidity of the vaccine spatial structure can also influence the immune response; viruses and VLPs have optimal sizes (20–200 nm), allowing them to enter the lymphatic system and be taken up by antigen presenting cells (APCs). To ensure efficient transport in the lymphatic system, massive aggregation of VLPs in vaccine preparations must be avoided;
3) Viruses and VLPs contain specific and/or host nucleic acids, which activate Toll-like receptors in antigen-presenting cells; if necessary, VLPs can be experimentally filled with the DNA or RNA of choice.

Several examples demonstrate the importance of antigen multiplicity in the generation of specific antibodies. Repeated administrations of potato virus X (PVX; [43]) lead to specific immune responses mediated by immunoglobulins M (IgM) and G (IgG); these responses result in the formation of large immune complexes containing aggregated VLPs at early stages of the response. Next, multiple antigens placed on the surface of PapMV VLPs stimulate a strong, long-lasting immune response, whereas monomeric PapMV CP-antigen fusion is not efficient despite the observed internalization of both model vaccines in APCs [44]. Moreover, the epitope density on the VLP surface plays an important role in the immunogenicity of plant VLP vaccines; the use of VLPs as a peptide carrier stimulates formation of the IgG2a isotype, whereas the peptide-KHL conjugate predominantly elicits the IgG1 subtype against model peptides [45]. In mice, it is well known that interferon-producing Th1 T cells stimulate antibody switch towards IgG2a, while Th2 cells promote IgG1 synthesis; IgG2a is the most potent antibody isotype against viral and bacterial pathogens and ensures rapid host immune responses to infections [46,47].

An additional aspect that should be considered in vaccine generation already in the construction stage is that viruses and VLPs, including those derived from plant virus CPs, can contain natural Th epitopes, which are presented on the surface of APCs by MHC presentation and can stimulate antibody generation [48]. The use of searchable databases, such as RANKPEP [49] or IEDB [50], can identify MHC binding AA motifs among MHC alleles of humans, chimpanzees, cows, pigs, mice and others. A bioinformatic approach can considerably facilitate the identification of potential vaccines [51] and enable early screening for viral structural proteins with high-binding affinity to several MHC alleles to cover a major population of potential recipients.

As we found in our literature search, plant viruses serve as central elements for at least 61 prophylactic and 12 therapeutic vaccine candidates (Supplement Tables 1 and 2). In this Chapter, we provide a short overview of genetic, enzymatic, chemical and physical approaches and how to obtain and adapt plant viruses and VLPs for vaccine applications. It should be stressed that these methods are frequently combined for the construction of VLPs with desired immunological properties. We also include here successful examples of other VLPs to demonstrate important construction principles, which can also be applied to plant VLPs in the future.

3.1. Genetic methods

Genetic techniques are powerful tools for creating virus-derived vaccines, allowing introduction of AA into VLP coats and inserting or replacing peptides, or even whole proteins, at defined CP positions. Moreover, these techniques allow better quantitative control of the amounts of introduced antigens than that of chemical techniques.

If natural CP sequences do not ensure the desired properties of target VLPs, AA introduction is frequently required. As discussed in the next Chapter, Lys- or Cys-residue insertions onto the VLP surface is necessary for subsequent chemical coupling [52]. Taking into account the importance of VLP stability, artificial Cys bridges can be engineered between neighboring viral CPs. The resulting VLPs demonstrate significantly enhanced self-assembly capability and stability under different environmental conditions [53].

As already discussed, the first recombinant peptide-VLP candidate vaccine has been obtained from TMV CP with a C-terminally fused poliovirus epitope. Since then, vaccine construction strategies have also included N-, C– and internal fusions of peptides to viral CPs. For vaccine construction, it is important to achieve optimal peptide presentation on the particle surface without affecting the overall viral structure. For peptide fusion, the availability of the virus spatial model is very helpful for the identification of insertion sites. For viruses such as TMV [54], PVX [55], PVY [56] and other helical viruses, the N- and/or C-terminal portions of CPs are located on the VLP surface, allowing insertion of antigenic peptide sequences. As a general rule, tolerated peptide sizes vary between 10 and 50 AA [37]; in exceptional cases, VLPs are able to accommodate more than 70 AA-long protein domains and retain the typical VLP morphology [56].

For icosahedral plant viruses, CP N-terminal portions are frequently located in the internal part of the virion and are involved in CP-nucleic acid interactions, which are usually necessary for VLP formation. However, some plant VLPs accept peptide fusions at the N- and C-termini of CPs and even the N-terminus removal, whereas the internal insertions interfere with VLP formation [57]. Other icosahedral plant viral particles, such as the well-studied CPMV, contain several internal sites for peptide insertions, such as βB-βC and βC′-βC″ loops of the S subunit and βE-αB loops of the L subunit [58]. It should be stressed that peptide properties, such as charge and hydrophobicity, influence VLP formation. For example, positively charged epitopes can have a negative impact on virion formation and virus infectivity in plant expression systems [59]; placing hydrophobic peptides such as the “padre” T-cell epitope onto the VLP surface significantly reduces the vaccine solubility [60]. However, specially designed charged linkers can help to improve the solubility and stability of foreign peptide-containing VLPs [61]. It should be added that the use of VLPs with a surface-engineered random peptide library is an attractive alternative for vaccine construction, as shown with bacteriophage PP7 VLPs [62].

It is known that T-cell activation involves a complex formation between major histocompatibility complex (MHC) and a corresponding

antigen peptide [63]. Targeted incorporation of peptides derived from well-characterized antigens in VLP vaccines results in strong T-cell stimulation and antibody formation [60,64], even under limiting conditions (low antigen doses, aged recipients, self-antigens), as shown with tetanus toxin peptide-containing plant VLPs derived from CMV [65].

Sometimes, peptide-VLP vaccines do not elicit the expected immune response and/or antibody specificity; altered peptide conformations or interactions with VLP carriers reduce the specificity of obtained antibodies against native antigens. Therefore, the introduction of protein domains, or even whole proteins, into the VLP structure can considerably improve the vaccine quality due to the closer spatial structure of the recombinant antigen to that found in the native protein. Direct fusions with whole proteins are challenging if the antigen sizes are comparable or even exceed that of viral CPs; successful examples are rare [66]. As a solution, several construction strategies are suggested for placing larger antigens onto the surface of plant VLPs. One solution is the construction of mosaic VLPs, containing a mixture of unchanged CPs and CPs with the foreign protein domain fusions in the same viral particle or VLP. As shown with infectious PVX in the plant expression system, the introduction of a self-processing peptide between the antigen sequence and the CP N-terminus results in filamentous VLPs that are partially decorated with the antigen ([67]; see also Fig. 3). Occasionally, the mosaic particles are formed after spontaneous proteolysis of the fusion protein during assembly in the host, resulting, for example,

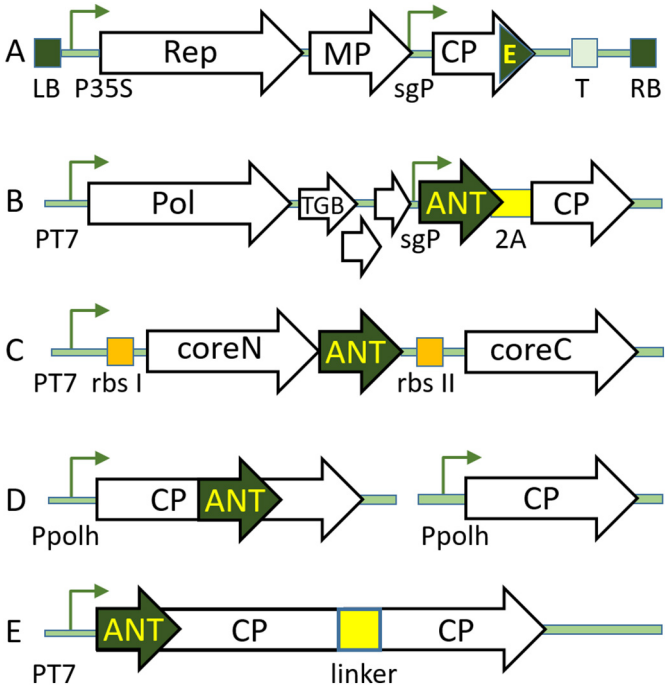

Fig. 3. . Design examples of gene vectors used for introduction of different antigens in viral structures. A – «launch» vector [106] based on TMV for production of peptide-containing viruses in plants. LB and RB denotes agrobacterial shuttle vector T-DNA region left and right borders; P35S – CaMV promoter; sgP – subgenomic promoter; T – transcription terminator; Rep, MP, CP – TMV replicase, movement protein and coat protein genes, respectively. Green arrow denotes the relative position of the inserted epitope (E). B – PVX-based vector for production of mosaic antigen (ANT)-containing viruses in plants [67]. PT7 – bacterial promoter for *in vitro* generation of infectious RNA; sgP – subgenomic promoter; 2A – self-processing peptide sequence; Pol, TGB, CP – polymerase, triple gene block and coat protein genes. C – Split-core system for incorporation of large antigens in VLP structure [69]. The antigen (ANT) gene is fused with coreN part of HBcAg and coexpressed with coreC part. After self-assembly of coreN and coreC parts, ANT is incorporated in VLP structure. PT7 – bacterial promoter for VLP production in E.coli; rbs – ribosomal binding sites. D – Two gene coexpression system [70] for production of mosaic VLPs containing antigens (ANT) in baculovirus/insect cell system. Ppolh – polyhedrin gene promoter. E – Tandem gene system for production of antigen-containing mosaic VLPs in *E.coli* cells [71]. PT7 – bacterial promoter; linker – flexible AA stretch connecting two CPs.

in 30% incorporation of the antigen into the VLP structure, as shown with the alfalfa mosaic virus (AlMV)-based antimalaria vaccine [68]. Other potentially applicable ideas for plant VLPs originate from nonplant VLP studies (see Fig. 3). Full-sized antigen presentation on the VLP surface is enabled by the split-core system [69]; mosaic VLPs with incorporated large antigens can be generated by targeted coexpression of two CP genes [70] and an in tandem gene system [71].

3.2. Chemical and enzymatic methods

Chemical conjugates between antigens and protein carriers are very useful for vaccine purposes, as shown more than 80 years ago when the immunostimulating properties of protein-coupled sugar derivatives were demonstrated. In the 1980s, this idea resulted in the creation of several vaccines against *Haemophylus influenza* (for review, see [72]).

The chemical coupling of peptides and protein domains with viruses and VLPs involves the use of different bifunctional crosslinking agents. Using crosslinkers, the amino-, sulfhydryl-, carboxyl- and other functional groups in the protein structure can be modified for coupling under specific reaction conditions (for reviews, see [73,74]). Some of these conditions are not compatible with preservation of the virus structure; therefore, process optimization is necessary to find the best reaction environment (pH, ionic strength, temperature, additives).

The primary amines of lysine residues located on the VLP surface are likely the most common coupling targets of different functional molecules with VLPs, including peptides and proteins. After reaction with different N-hydroxysuccinimide (NHS) esters, these amines form stable amide-bonds (Fig. 4). This method is widely used for fluorescent labeling and PEGylation of plant viruses [75] and for the construction of several model vaccines [36], including those derived from plant VLPs [65,76,77].

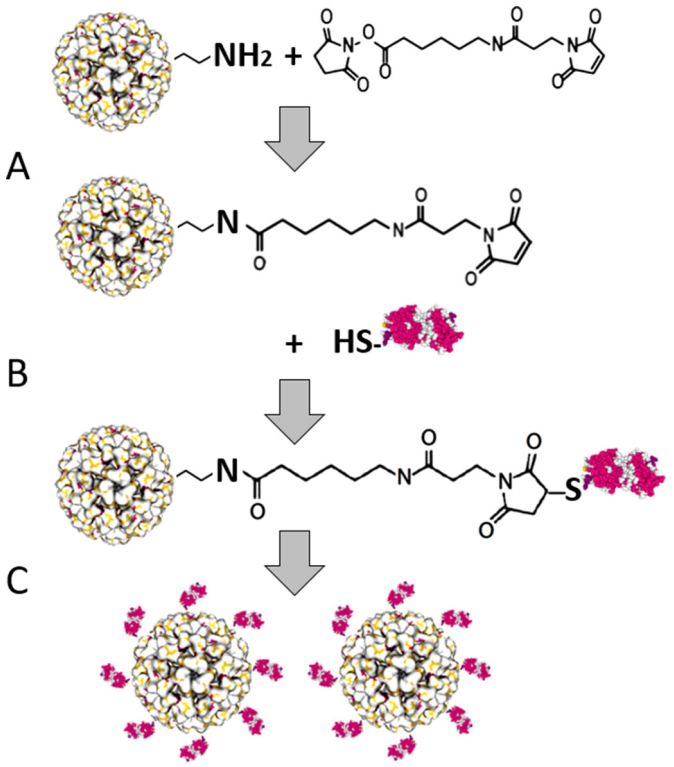

Fig. 4. Antigen incorporation in plant virus structure by chemical coupling. Primary amines of free accessible Lys residues are reacting with heterobifunctional crosslinking reagent SMPH (succinimidyl-6-[(β-maleimidopropionamido)hexanoate]), forming stable amide bound (A). In the next step (B), the thiol group in the Cys-containing antigen structure selectively reacts with the maleimide derivative, resulting in VLPs decorated with multiple antigen molecules (C).

Viruses and VLPs may also contain freely accessible carboxylic groups, which can be used for antigen coupling. As a recent example, CCMV plant virus has 1980 carboxylate residues on the particle surface, which can be activated by water-soluble carbodiimide. A subsequent reaction with NHS results in succinimide-esterified CCMV VLPs, which can form an amide bond with molecules containing a primary amine group [78].

Sulfhydryl groups of natural and engineered cysteine residues in viral and VLP structures can also be useful for antigen coupling. As shown in a recent study with a TMV-Cys mutant, free sulfhydryl groups actively react with maleimide derivatives, forming stable thioether linkages [79]. The use of the corresponding maleimide-containing bifunctional linker results in the covalent bond between sulfhydryls in recombinant plant viruses and amine groups of the chosen ligand.

To complete the discussion regarding chemical and enzymatic approaches for synthesis of antigen-VLP conjugates, it is important to mention two additional recently developed systems based on transpeptidase and isopeptidase reactions (see also Fig. 5):

1) Sortase A-mediated epitope coupling system involves the use of bacterial transpeptidase for formation of an amide bond between VLP and a peptide of interest; papaya mosaic virus (PapMV) VLPs with an engineered sortase recognition site can be useful as a vaccine platform [80];
2) Spy-Catcher system originates from *Streptococcus pyogenes* bacterium, which exploits peptides that are able to spontaneously form isopeptide bonds; the Spy-Tag-conjugated antigens form covalent bonds with Spy-Catcher-VLPs derived from the bacteriophage AP205 [81].

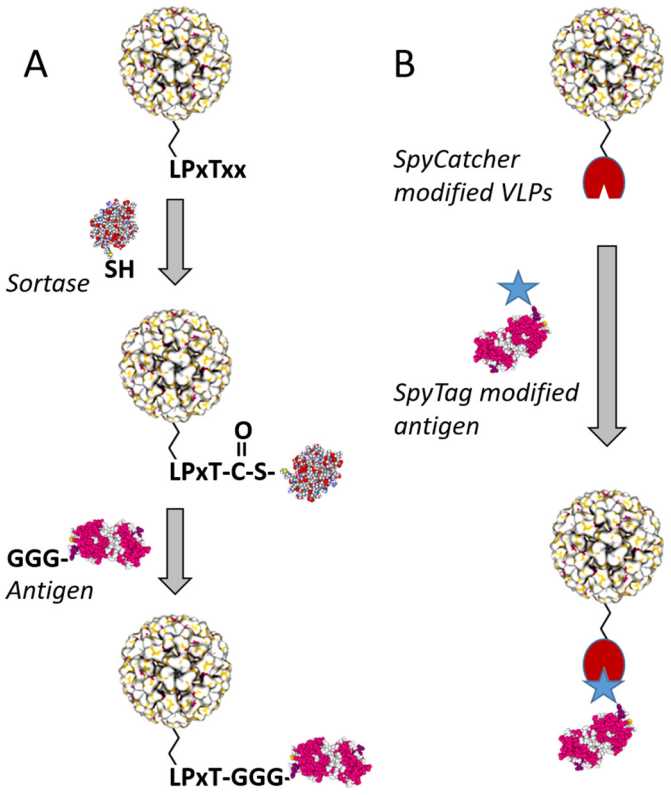

Fig. 5. Enzymatic approaches for synthesis of antigen-VLP conjugates. A – Sortase A mediated coupling of VLPs to antigens [80,150]; B – Spy-Catcher system for covalent binding of SpyTag conjugated antigens to VLPs containing exposed SpyCatcher domain [81,151]

3.3. Physical methods

An important physical manipulation in vaccine development is the disassembly and reassembly process, which is based on the intrinsic property of viral structural proteins to self-assemble under optimal conditions. This process can be used for improvement of VLP structural properties and for the introduction of chosen antigens in the VLP structure. The reassembly approach was very efficient in the production of prophylactic vaccines against cervical cancer. As parameters of ionic strength, pH and other factors in heterologous host cells are typically different from those in the native host, VLPs can have reduced immunogenicity and antigenicity properties. Therefore, after expression in the baculovirus/insect cell system, recombinant HPV L1 protein oligomers are purified and assembled *in vitro*. Resulting VLP structures are identical to those of native virions, including spatial arrangement of conformational epitopes, which are necessary for the formation of neutralizing antibodies. Additionally, the reassembly process ensures the required batch-to-batch consistency and VLP stability under different conditions, including vaccine formulations with adjuvants [82]. The disassembly/reassembly strategy is also used in preparations of the hepatitis E vaccine: *E. coli*-produced inclusion bodies of CPs are solubilized in concentrated urea solution, purified under denaturing conditions and refolded by tangential filtration [83].

Similar to other VLPs, plant viral CPs can be produced in the form of inclusion bodies in *E. coli* cells, refolded, assembled in the form of VLPs and used for different nanotechnological applications, including vaccines. Cowpea chlorotic mottle virus (CCMV) is a typical example that serves as a model nanoparticle in numerous studies (see CCMV references in Supplement Table 3). CCMV CPs are able to form VLPs after purification under denaturing conditions and the reassembly procedure, including genetically modified CPs, which contain foreign epitopes derived from human or animal viruses (for example, [57]).

Additionally, the disassembly/reassembly procedure is very useful when the intended application requires the packaging of chosen active molecules, including nucleic acids in the interior of VLPs. To remove specific nucleic acids from virions, viral particles are disassembled in high-salt buffer and reassembled in the presence of short oligonucleotides [84]. Interestingly, this procedure can pave the way for manipulations with VLP morphology, converting icosahedral VLPs to rod-shaped particles, as shown in DNA packaging experiments with Cucumber mosaic virus (CMV) [85]. Using viruses and VLPs filled with host RNAs or artificially loaded with unmethylated DNA oligonucleotides, model vaccines stimulate Toll-like receptors (TLR7/8 and TLR9) in APCs that results in antibody isotype switching to IgG2a [47,86]. As discussed in [87], vaccination with PVX particles containing infectious RNA results in enhanced IL12 production by dendritic cells, which further activate CD4 Th1 cells. Additionally, packaged nucleic acid can stimulate TLR receptors directly in B cells, resulting in antibody switching to IgG2a. The stimulation of Th1 cells and isotype switching have also been shown for other vaccine candidates derived from plant viruses, such as CPMV [88], PapMV [89] and tobacco etch virus [90].

Other physical manipulations with plant virus-derived particles can be useful for vaccine construction. For TMV, thermal treatment of viral particles results in transformation of rods into spherical particles that are applicable as potential vaccines [91]. Additionally, after purification from recombinant bacterial cells, some truncated plant viral CPs are able to form smaller VLPs ($T = 1$ symmetry instead of $T = 3$) [92]. These experiments demonstrate ideas of how to adapt vaccine carriers to the above-discussed size requirements of efficient VLP vaccines.

Full-sized viral structural proteins usually serve as building blocks for VLPs. However, comparably short structural elements of different CPs can promote the multimerization of model proteins, as previously shown with TBSV and SeMV plant viruses. In these viruses, specific internally located CP domains interact with each other and stabilize the virion structure. Furthermore, these domains are sufficient to mediate the formation of large protein assemblies up to sizes of typical VLPs, if

genetically fused to antigens of choice. The addition of such viral CP fragments to the antigen proteins can have an application in vaccine construction [93,94].

Different physical techniques can be used for the introduction of peptides and protein domains into VLP-based nanoparticles. The interaction between streptavidin and biotin is one of the strongest protein-molecule complexes in nature. As previously demonstrated with modified TMV [95] and PVX [76], the streptavidin-biotin complex strategy allows simple introduction of different antigens, including large proteins that exceed the size limitations of genetically introduced antigens.

Additionally, the strong antigen binding to the VLP carrier has been demonstrated using other noncovalent complexes, such as strong electrostatic interactions between VLPs and antigen [96] and the introduction of the *S. aureus* protein A fragment into VLPs for antibody binding [97,98]. Other methods include the inclusion of elastin-like [99] and coiled-coil motifs into plant VLP structures [100], antigen-peptide complex formation after library screening [101] and simple absorption of antigen onto the VLP surface [91]. Additionally, a recent study suggested that a simple mixture of different VLPs reaches the lymph nodes in less than 10 min and is processed by the same antigen-presenting cells (APCs). This finding suggests that mixtures of VLPs can also be used in vaccination [102].

3.4. Expression systems

The production of viral material in preparative amounts is an important task during the development of VLP vaccines. Plant viruses are generally recognized as safe for humans and animals, and this aspect will be discussed in Chapter 4. Therefore, native plant viruses are promising objects for vaccine development, especially due to the availability of some plant viruses in large quantities. Plant viruses, such as TMV, PVX, CPMV and CCMV, are highly stable at elevated temperatures, pressure and pH conditions and can be purified from their native plant hosts in quantities exceeding hundreds of mg/kg plant biomass (reviewed in [74]). The use of native viruses facilitates the vaccine construction process and excludes the requirement for viral gene cloning and elaborate heterologous expression systems. However, native and recombinant plant VLPs may contain infectious nucleic acids. Therefore, additional virus inactivation step(s) are included in the production schemes to reduce potential environmental risks [103].

For vaccine purposes, significant portions of the plant virus infectious particles and VLPs are produced in different plant systems; out of 72 cases discussed in this review, 49 (68%) vaccine candidates originated from plants (see Supplement Tables 1 and 2).

As discussed earlier, plants can be artificially infected by simple *in vitro*-prepared transcripts derived from the full cDNA of a plant virus [104,105]. However, this approach is inefficient due to RNA vector instability and low production levels. More effective plant systems are based on the agrobacterial transfer of viral nucleic acids into the plant cell nucleus, where viral transcripts are synthesized and translocated to the cytosol for the initiation of infection. Moreover, the plant cells become up to 100% infected if agrobacteria carrying virus cDNA are transferred into plant cells by vacuum infiltration. Based on this principle, two efficient systems have been constructed – the "launch" vector ([106]; Fig. 3A) and "magnifection" system [107]. Both systems allow production of different recombinant proteins, including vaccines with a high output. Additional information about developed plant expression systems can be found in recent review articles [108,109].

Many (30%) plant VLP-based vaccines are produced in well-characterized *E. coli* expression strains using commercially available plasmid vectors (see Supplement Tables 1 and 2). Typically, eukaryotic proteins, including structural proteins of plant viruses, form insoluble inclusion bodies after synthesis in *E. coli*. The VLP production from inclusion bodies requires purification under denaturing conditions and *in vitro* self-assembly, as shown with plant VLPs derived from CCMV and CMV [85,110]. However, our recent study demonstrated that simple temperature reduction during the recombinant *E. coli* cultivation considerably enhances the formation of CMV VLPs in a soluble form and enables VLP production with high output [65]. For production of soluble CCMV VLPs, an alternative *Pseudomonas*-based bacterial system is suggested [111].

Yeast expression systems have traditionally been used in vaccine production since the first commercial VLP vaccine against HBV. Some plant VLPs are produced in yeast *Pichia pastoris*, which allows VLPs to be obtained in soluble form [112].

Plant VLPs are also produced in insect cells using baculovirus vectors. To elucidate the mechanism of virus formation, CPMV RNA-2-encoded precursor proteins have been expressed in insect cells, resulting in formation of CPMV-like particles [113]. For vaccine purposes, the TBSV epitope display system has been developed based on baculovirus-mediated expression in insect cells [114].

Additionally, mammalian cells can also be useful for plant VLP production [115].

The advantages, drawbacks and other aspects of VLP vaccine production in different recombinant hosts are extensively discussed in several review articles [15,74,116,117].

3.5. Plant virus-derived platforms

The concept of a platform can be defined as a complex of technology measures that are used for creation, production and quality evaluation of a group of marketable products with similar properties or modes of action. The platform idea includes several requirements, such as broad applicability, addressing a wide spectrum of problems; platforms represent alternatives to existing processes and are efficient and scalable [118].

For viral particle- and VLP-based vaccine platforms, virus-derived structures are the central elements of the platform. We have already discussed the main properties of viral particles and VLPs, which ensure their broad applicability as vaccines, such as antigen multiplicity and structural organization, particle dimensions and presence of encapsidated nucleic acids. To generate vaccines against different targets based on the same viral or virus-like particle, the creation process includes linking the VLP carrier with corresponding antigens using physical, chemical and/or genetic methods, as discussed in previous chapters. The principles of modular vaccine design and manufacturing as well as the advantages of the platform technologies in vaccine production and marketing are described in a recent review article [119].

Plant virus- and VLP-based vaccines meet all these criteria, including additional important advantages, such as low risk of preexisting immunity, excellent stability properties under different environmental conditions, simple engineering procedures and low-cost production [37]. There are several examples of plant viruses that have been developed as vaccine platforms, such as AlMV, CMV, CPMV, PapMV, PVX, and TMV (for additional information and references, see Supplement Table 3).

Several vaccines obtained from plant virus platforms have been analyzed for tolerability, safety and efficacy in human clinical studies. One of the first plant virus-based vaccines is an edible vaccine in the form of spinach leaves containing recombinant the antigenic determinant of rabies virus fused to AlMV. Although the vaccine efficacy in Phase I clinical trial has not been sufficiently high for further developments, the most important finding of this study is the lack of any adverse effects in human volunteers after oral immunization with rabies antigen-containing spinach plants [120].

Additionally, two other plant virus-derived vaccines are currently being evaluated in human trials. Based on the same AlMV platform, a recent clinical Phase I study of a Pfs25-based malaria vaccine has demonstrated acceptable tolerability and moderate transmission-blocking activity of induced antibodies in human volunteers. Using alternative antigens from *Plasmodium falciparum,* a new version of the vaccine is under development [121,122]. The second human trial is still ongoing

and aims to assess the safety and reactogenicity of PapMV VLPs as the adjuvant for use in the seasonal flu trivalent vaccine in healthy individuals [123].

Taking into account the excellent immunological properties of plant virus-based vaccines, these are also being evaluated in veterinary trials. As one such vaccine, the CMV-based porcine circovirus (PCV) peptide vaccine is safe, immunogenic and confers partial protection against viral challenge. Additionally, this study demonstrated the favorable internal sites for peptide insertions in the structure of CMV CP, allowing optimal display of the peptides on the particle surface [124]. The next vaccine based on CPMV protects vaccinated dogs against a lethal challenge of canine parvovirus. To exclude the spread of infectious plant viruses in the environment, recombinant CPMV virions can be inactivated by UV treatment without a loss of immunogenicity [125]. Another vaccine against foot and mouth disease virus (FMDV) is designed on bamboo mosaic virus (BaMV); the vaccine protects animals against FMDV infection [126].

Recently, we constructed a new vaccine platform based on the CMV plant VLPs [65]. First, we demonstrated that the internally located N-terminal part of CMV CP can be replaced by a well-characterized T-cell stimulating epitope derived from tetanus toxin without affecting the VLP morphology. Next, Lys residues exposed on the VLP surface enable the linkage of any antigen of choice to VLPs *via* chemical crosslinking. Accordingly, our vaccine platform represents genetically modified CMV-VLPs that are produced in *E. coli*; the necessary native or recombinant antigens are coupled to the VLP surface using bifunctional chemical linkers. The chosen modular strategy allows us to create various prophylactic and therapeutic vaccines against different infectious agents and self-antigens. Using this platform, we generated several therapeutic veterinary vaccines displaying several antigens, including canine IL31 [127] and horse IL5 [128], on the surface of CMV VLPs for the treatment of atopic dermatitis in dogs and insect bite hypersensitivity in horses. As found in veterinary clinical experiments, both vaccines are well tolerated and reduce the symptoms in vaccinated dogs and horses.

4. Plant viruses and mammalian organisms

It is generally recognized that plant viruses are not infectious to mammalian organisms, including humans. However, plants used in human and animal nutrition can contain viruses. In recent years, several research groups have studied the fate of plant viruses in mammalian organisms. In one such study, a metagenomic analysis of uncultured RNA viruses in the gastrointestinal tract of healthy humans, resulted in a surprising outcome: researchers found that a significant portion of isolated RNA populations represent RNA from 34 different plant viruses instead of the expected human enteric viruses. Moreover, pepper mild mottle virus (PMMoV) isolated from human feces is infectious to susceptible plant hosts. The obtained data suggest that plant viruses are stable in the gastrointestinal tract, and humans are the dissemination vehicles for spread of some plant viruses in nature [129]. Subsequent studies renewed discussions concerning the infectivity of plant viruses in mammalian organisms and explained the antibody formation against PPMoV in human patients as possible result of virus pathogenicity [130]. However, other authors suggest that the “pathogenicity” effect is a result of PPMoV's extreme environmental stability [23].

The ideas of edible vaccines originating directly from plant biomass [131] and the plant virus-based “new agriculture” [132] have significantly stimulated studies aimed at elucidating the relationships between plant viruses and mammalian organisms. The CPMV plant virus has been shown to remain structurally stable after incubation in simulated gastric and intestinal fluids containing digestive enzymes. Moreover, fluorescently labeled CCMV and CPMV demonstrate the ability of systemic distribution in different organs of experimental animals (brain, bone marrow, liver, lungs, spleen, stomach) independently of the administration route. However, viruses are rapidly cleared from mice within several days [133,134]. Toxicology studies suggest that large doses of injected plant viruses do not cause significant necroses, tissue degeneration or hemolysis in tested animals [135,136]. As reviewed in [137], CPMV interacts with specific mammalian cell targets, facilitating virus uptake; other plant viruses enter the cells *via* nonspecific or unknown mechanisms, and the effect can be reduced using PEGylated viruses.

If we analyze vaccine injections in mammalian organisms, the initial events are interactions between plasma proteins and vaccine antigens. The multimeric structure of viruses and VLPs stimulates spontaneous deposition of complement proteins on the foreign agent surface, leading to the opsonization and activation of APCs [41,138]. As shown in experiments with TMV-derived nanoparticles, TMV virions are able to bind up to 140 plasma proteins, and a significant portion of them is immunoglobulins. Interestingly, the pattern and amounts of bound complement proteins can be manipulated by changing the surface charge or by chemical coupling of peptides and PEGs [139]. As shown in another study, the role of native antibodies and complement proteins in plant VLP recognition and phagocytosis can be different from that found in experiments with infectious mammalian viruses [140].

Another aspect that should discussed is molecular mimicry, suggesting that a foreign agent-like plant virus can share antigens with the heterologous host. As a result, these antigens can be involved in autoimmune reactions and protect individuals against diseases. A recent study demonstrated interesting properties of antibodies against TMV, possibly preventing smokers from Parkinson's disease; other plant viruses may also contain epitopes that allow interactions with mammalian cells. Therefore, before introducing a new proteinaceous drug, including vaccines, the search for homologous AA sequences in recipients should be carried out to predict unexpected immune responses [141].

5. Concluding remarks

In this review, we have summarized the publicly available data about plant virus-derived vaccines and have discussed the main aspects of efficient vaccine construction. Plant viruses, along with other virus-like carriers, are highly promising agents for antigen presentation, stimulating the response of different immune cells and eliciting the formation of specific antibodies against infectious and self-antigen targets, as has been demonstrated in numerous studies. Plant VLP-based vaccines have additional advantages, such as uncomplicated and flexible techniques of vaccine construction, simple procedures for virus and VLP production and purification, remarkable stability properties and a low possibility of preexisting immunity. All these advantages make plant viruses an attractive alternative to animal and human VLPs.

Several plant viruses and VLPs have been constructed based on vaccine platform principles, allowing scientists to obtain vaccines against different targets using the same plant virus-based carrier.

One of the most important problems yet to be solved during the vaccine construction process is antigen spatial conformation. The chosen experimental methods for the incorporation of recombinant antigens into VLPs must ensure a maximal structural equivalence to their native analogs. First, different computer-based modeling methods have been suggested [142]. Next, the construction process of an efficient vaccine is considerably facilitated if several available VLP platforms are compared. Such an approach can improve the quality of antibodies, as shown with the PVX- and CMV-derived anti-HCV vaccines [143,144]; other examples of the same antigen displayed on different carriers can be found in Supplement Tables 1 and 2. Moreover, the same principle can be applied to the method used for antigen presentation on the VLP surface: comparisons of physical mixture, chemical coupling and genetic fusions are useful in choosing the optimal strategy for subsequent VLP vaccine development [102,145,146].

Future viral particle and VLP vaccines will be more sophisticated and will contain antigens arranged in multimeric arrays on carrier surfaces, different immunostimulating components, such as special nucleic acids,

and well-characterized T-cell epitopes. The experience gained from other virus-like vaccines is very valuable and will promote the construction of more universal plant VLPs. The achievements in plant virus-based vaccine development suggest that native plant viruses and their recombinant derivatives are fully adaptable to vaccine requirements and will serve as central components of new prophylactic and therapeutic vaccines for broad applications in humans and animals in the near future.

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Appendix A. Supplementary data

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References

[1] F.E. Andre, R. Booy, H.L. Bock, J. Clemens, S.K. Datta, T.J. John, B.W. Lee, S. Lolekha, H. Peltola, T.A. Ruff, M. Santosham, H.J. Schmitt, Vaccination greatly reduces disease, disability, death and inequality worldwide, Bull. World Health Organ. 86 (2008) 81–160.
[2] S. Plotkin, History of vaccination, Proc. Natl. Acad. Sci. U. S. A. 111 (2014) 12283–12287.
[3] S.A. Plotkin, Vaccines: past, present and future, Nat. Med. 11 (2005) S5–11.
[4] P. Valenzuela, A. Medina, W.J. Rutter, G. Ammerer, B.D. Hall, Synthesis and assembly of hepatitis B virus surface antigen particles in yeast, Nature 298 (1982) 347–350.
[5] M.R. Hilleman, W.J. McAleer, E.B. Buynak, A.A. McLean, AA the preparation and safety of hepatitis B vaccine, J. Inf. Secur. 7 (1983) 3–8.
[6] B.J. Cohen, J.E. Richmond, Electron microscopy of hepatitis B core antigen synthesized in *E. coli*, Nature 296 (1982) 677–678.
[7] A.D.T. Barret, Vaccinology in the twenty-first century, Vaccines 1 (2016) 16009.
[8] D. Shouval, H. Roggendorf, M. Roggendorf, Enhanced immune response to hepatitis B vaccination through immunization with a Pre-S1/pre-S2/S vaccine, Med. Microbiol. Immunol. 204 (2015) 57–68.
[9] D.R. Lowy, J.T. Schiller, Prophylactic human papillomavirus vaccines, J. Clin. Invest. 116 (2006) 1167–1173.
[10] E. Petrosky, J.A. Bocchini, S. Hariri, H. Chesson, C.R. Curtis, M. Saraiya, E.R. Unger, L.E. Markowitz, Use of 9-valent human papillomavirus (HPV) vaccine: updated HPV vaccination recommendations of the advisory committee on immunization practices, MMWR Morb. Mortal. Wkly Rep. 64 (2015) 300–304.
[11] J. Paavonen, P. Naud, J. Salmerón, C.M. Wheeler, S.N. Chow, D. Apter, H. Kitchener, X. Castellsague, J.C. Teixeira, S.R. Skinner, J. Hedrick, U. Jaisamrarn, G. Limson, S. Garland, A. Szarewski, B. Romanowski, F.Y. Aoki, T.F. Schwarz, W.A. Poppe, F.X. Bosch, D. Jenkins, K. Hardt, T. Zahaf, D. Descamps, F. Struyf, M. Lehtinen, G. Dubin, Efficacy of human papillomavirus (HPV)-16/18 AS04-adjuvanted vaccine against cervical infection and precancer caused by oncogenic HPV types (PATRICIA): final analysis of a double-blind, randomised study in young women, Lancet 374 (2009) 301–314.
[12] X. Zhang, M. Wei, H. Pan, Z. Lin, K. Wang, Z. Weng, Y. Zhu, L. Xin, J. Zhang, S. Li, N. Xia, Q. Zhao, Robust manufacturing and comprehensive characterization of, recombinant hepatitis E virus-like particles in Hecolin, Vaccine 32 (2014) 4050.
[13] RTSS Clinical Trials Partnership, Efficacy and safety of RTS, S/AS01 malaria vaccine with or without a booster dose in infants and children in Africa: final results of a phase 3, individually randomised, controlled trial, Lancet 6736 (2015) 31–45.
[14] K.A. Collins, R. Snaith, M.G. Cottingham, S.C. Gilbert, A.V.S. Hill, Enhancing protective immunity to malaria with a highly immunogenic virus-like particle vaccine, Sci. Rep. 7 (2017), 46621. .
[15] X. Huang, X. Wang, J. Zhang, N. Xia, Q. Zhao, Escherichia coli-derived virus-like particles in vaccine development, Vaccines 2 (2017) 3.
[16] M. Chow, R. Yabrov, J. Bittle, J. Hogle, D. Baltimore, Synthetic peptides from four separate regions of the poliovirus type 1 capsid protein VP1 induce neutralizing antibodies, Proc. Natl. Acad. Sci. U. S. A. 82 (1985) 910–914.
[17] R.A. Lerner, N. Green, H. Alexander, F.T. Liu, J.G. Sutcliffe, T.M. Shinnick, Chemically synthesized peptides predicted from the nucleotide sequence of the hepatitis B virus genome elicit antibodies reactive with the native envelope protein of Dane particles, Proc. Natl. Acad. Sci. U. S. A. 78 (1981) 3403–3407.
[18] J.R. Haynes, J. Cunningham, A. von Seefried, M. Lennick, R.T. Garvin, S.H. Shen, Development of a genetically-engineered, candidate polio vaccine employing the self assembling properties of the tobacco mosaic virus coat protein, Biotechnology 4 (1986) 637–641.
[19] B.D. Harrison, T.M.A. Wilson, Milestones in the research on tobacco mosaic virus, Phil. Trans. R. Soc. Lond. B. 354 (1999) 521–529.
[20] Y. Okada, Historical overview of research on the tobacco mosaic virus genome: genome organization, infectivity and gene manipulation, Phil. Trans. R. Soc. Lond. B 354 (1999) 569–582.
[21] M. Zaitlin, P. Palukaitis, Advances in understanding plant viruses and virus diseases, Annu. Rev. Phytopathol. 38 (2000) 117–143.
[22] K.B. Scholthof, S. Adkins, H. Czosnek, P. Palukaitis, E. Jacquot, T. Hohn, B. Hohn, K. Saunders, T. Candresse, P. Ahlquist, C. Hemenway, G.D. Foster, Top 10 plant viruses in molecular plant pathology, Mol. Plant Pathol. 12 (2011) 938–954.
[23] M.J. Roossinck, Plant Virus Ecology, PLoS Pathog. 9 (2013), e1003304. .
[24] S.G. Lazarowitz, Plant viruses, in: D.M. Knipe, P.M. Howley (Eds.), Fundamental Virology, 4th ednLippincot, Williams & Williams, Philadelphia 2001, pp. 377–442.
[25] W.A. Miller, K.A. White, Long-distance RNA-RNA interactions in plant virus gene expression and replication, Annu. Rev. Phytopathol. 44 (2006) 447–467.
[26] A. Zeltins, Plant virus biotechnology platforms for expression of medicinal proteins, in: Y.E. Khudyakov (Ed.), Medicinal Protein Engineering, CRC Press, Boca Raton 2009, pp. 481–517.
[27] A. Gierer, G. Schramm, G. Infectivity of ribonucleic acid from tobacco mosaic virus, Nature 177 (1956) 702–703.
[28] P. Goelet, G.P. Lomonossoff, P.J.G. Butler, M.E. Akam, M.J. Gait, J. Karn, Nucleotide sequence of tobacco mosaic virus RNA, Proc. Natl. Acad. Sci. U. S. A. 79 (1982) 5818–5822.
[29] P. Ahlquist, R. French, M. Janda, L.S. Loesch-Fries, Multicomponent RNA plant virus infection derived from cloned viral cDNA, Proc. Natl. Acad. Sci. U. S. A. 81 (1984) 7066–7070.
[30] S.C. Harrison, A.J. Olson, C.E. Schutt, F.K. Winkler, G. Bricogne, Tomato bushy stunt virus at 2.9 A resolution, Nature 276 (1978) 368–373.
[31] K. Namba, G. Stubbs, Structure of tobacco mosaic virus at 3.6A resolution: implications for assembly, Science 231 (1986) 1401–1406.
[32] X. Agirrezabala, E. Méndez-López, G. Lasso, M.A. Sánchez-Pina, M. Aranda, M. Valle, The near-atomic cryoEM structure of a flexible filamentous plant virus shows homology of its coat protein with nucleoproteins of animal viruses, elife 4 (2015) e11795.
[33] S. Yang, T. Wang, J. Bohon, M.E. Gagné, M. Bolduc, D. Leclerc, H. Li, Crystal structure of the coat protein of the flexible filamentous papaya mosaic virus, J. Mol. Biol. 422 (2012) 263–273.
[34] T.F. Anderson, W.M. Stanley, A study by means of the electron microscope of the reaction between tobacco mosaic virus and its antiserum, J. Biol. Chem. 139 (1941) 339–344.
[35] M.F. Bachmann, F. Zabel, Immunology of virus-like particles, in: Y.E. Khudyakov, P. Pumpens (Eds.), Viral Nanotechnology, CRC Press, Boca Raton 2016, pp. 121–128.
[36] G.T. Jennings, M.F. Bachmann, Immunodrugs: therapeutic VLP-based vaccines for chronic diseases, Annu. Rev. Pharmacol. Toxicol. 49 (2009) 303–326.
[37] M.E. Lebel, K. Chartrand, D. Leclerc, A. Lamarre, Plant viruses as nanoparticle-based vaccines and adjuvants, Vaccines (Basel) 3 (2015) 620–637.
[38] N. Benne, J. van Duijn, J. Kuiper, W. Jiskoot, B. Slütter, Orchestrating immune responses: how size, shape and rigidity affect the immunogenicity of particulate vaccines, J. Control. Release 234 (2016) 124–134.
[39] T.J. Moyer, A.C. Zmolek, D.J. Irvine, Beyond antigens and adjuvants: formulating future vaccines, J. Clin. Invest. 126 (2016) 799–808.
[40] M.O. Mohsen, L. Zha, G. Cabral-Miranda, M.F. Bachmann, Major findings and recent advances in virus-like particle (VLP)-based vaccines, Semin. Immunol. 34 (2017) 123–132.
[41] A.C. Gomes, M. Mohsen, M.F. Bachmann, Harnessing nanoparticles for immunomodulation and vaccines, Vaccines (Basel) 5 (2017) E6.
[42] M.F. Bachmann, G.T. Jennings, Vaccine delivery: a matter of size, geometry, kinetics and molecular patterns, Nat. Rev. Immunol. 10 (2010) 787–796.
[43] S. Shukla, R.D. Dorand, J.T. Myers, S.E. Woods, N.M. Gulati, P.L. Stewart, U. Commandeur, A.Y. Huang, N.F. Steinmetz, Multiple administrations of viral nanoparticles alter in vivo behavior-insights from intravital microscopy, ACS Biomater. Sci. Eng. 2 (2016) 829–837.
[44] J. Denis, N. Majeau, E. Acosta-Ramirez, C. Savard, M.C. Bedard, S. Simard, K. Lecours, M. Bolduc, C. Pare, B. Willems, N. Shoukry, P. Tessier, P. Lacasse, A. Lamarre, R. Lapointe, C. Lopez Macias, D. Leclerc, Immunogenicity of papaya mosaic virus-like particles fused to a hepatitis C virus epitope: evidence for the critical function of multimerization, Virology 363 (2007) 59–68.
[45] B.L. Nicholas, F.R. Brennan, J.L. Martinez-Torrecuadrada, J.I. Casal, W.D. Hamilton, D. Wakelin, D. Characterization of the immune response to canine parvovirus induced by vaccination with chimaeric plant viruses, Vaccine 20 (2002) 2727–2734.
[46] E.K. Deenick, J. Hasbold, P.D. Hodgkin, Decision criteria for resolving isotype switching conflicts by B cells, Eur. J. Immunol. 35 (2005) 2949–2955.
[47] A. Jegerlehner, P. Maurer, J. Bessa, H.J. Hinton, M. Kopf, M.F. Bachmann, TLR9 signaling in B cells determines class switch recombination to IgG2a, J. Immunol. 178 (2007) 2415–2420.
[48] K.M. Frietze, D.S. Peabody, B. Chackerian, Engineering virus-like particles as vaccine platforms, Curr. Opin. Virol. 18 (2016) 44–49.
[49] P.A. Reche, E.L. Reinherz, Prediction of peptide-MHC binding using profiles, Methods Mol. Biol. 409 (2007) 185–200.
[50] I. Hoof, B. Peters, J. Sidney, L.E. Pedersen, A. Sette, O. Lund, S. Buus, M. Nielsen, NetMHCpan, a method for MHC class I binding prediction beyond humans, Immunogenetics 61 (2009) 1–13.
[51] R.E. Soria-Guerraa, R. Nieto-Gomez, D.O. Govea-Alonso, S. Rosales-Mendoza, An overview of bioinformatics tools for epitope prediction: Implications on vaccine development, J. Biomed. Inform. 53 (2015) 405–414.
[52] F.C. Geiger, F.J. Eber, S. Eiben, A. Mueller, H. Jeske, J.P. Spatz, C. Wege, TMV nanorods with programmed longitudinal domains of differently addressable coat proteins, Nanoscale 5 (2013) 3808–3816.

[53] K. Zhou, F. Li, G. Dai, C. Meng, Q. Wang, Disulfide bond: Dramatically enhanced assembly capability and structural stability of tobacco mosaic virus nanorods, Biomacromolecules 14 (2013) 2593–2600.
[54] M. Koo, M. Bendahmane, G.A. Lettieri, A.D. Paoletti, T.E. Lane, J.H. Fitchen, M.J. Buchmeier, R.N. Beachy, Protective immunity against murine hepatitis virus (MHV) induced by intranasal or subcutaneous administration of hybrids of tobacco mosaic virus that carries an MHV epitope, Proc. Natl. Acad. Sci. USA 96 (1999) 7774–7779.
[55] C.C. Lico, P. Mancini, C. Italiani, C. Betti, D. Boraschi, E. Benvenuto, S. Baschieri, Plant-produced potato virus X chimeric particles displaying an influenza virus-derived peptide activate specific CD8+ T cells in mice, Vaccine 27 (2009) 5069–5076.
[56] I. Kalnciema, D. Skrastina, V. Ose, P. Pumpens, A. Zeltins, Potato virus Y-like particles as a new carrier for the presentation of foreign protein stretches, Mol. Biotechnol. 52 (2012) 129–139.
[57] A. Hassani-Mehraban, S. Creutzburg, L. van Heereveld, R. Kormelink, Feasibility of Cowpea chlorotic mottle virus-like particles as scaffold for epitope presentations, BMC Biotechnol. 15 (2015) 80.
[58] L. Liu, M.C. Canizares, W. Monger, Y. Perrin, E. Tsakiris, C. Porta, N. Shariat, L. Nicholson, G.P. Lomonossoff, Cowpea mosaic virus-based systems for the production of antigens and antibodies in plants, Vaccine 23 (2005) 1788–1792.
[59] C. Porta, C.V.E. Spall, K.C. Findlay, R.C. Gergerich, C.E. Farrance, G.P. Lomonossoff, Cowpea mosaic virus-based chimaeras. Effects of inserted peptides on the phenotype, host range, and transmissibility of the modified viruses, Virology 310 (2003) 50–63.
[60] A. Gedvilaite, I. Kucinskaite-Kodze, R. Lasickiene, A. Timinskas, A. Vaitiekaite, D. Ziogiene, A. Zvirbliene, Evaluation of *Trichodysplasia* Spinulosa-associated polyomavirus capsid protein as a new carrier for construction of chimeric virus-like particles harboring foreign epitopes, Viruses 7 (2015) 4204–4229.
[61] A. Tekewe, N.K. Connors, A.P. Middelberg, L.H. Lua, Design strategies to address the effect of hydrophobic epitope on stability and in vitro assembly of modular virus-like particle, Protein Sci. 25 (2016) 1507–1516.
[62] C. Caldeira Jdo, A. Medford, R.C. Kines, C.A. Lino, T.T. Schiller, B. Chackerian, D.S. Peabody, Immunogenic display of diverse peptides, including a broadly cross-type neutralizing human papillomavirus L2 epitope, on virus-like particles of the RNA bacteriophage PP7, Vaccine 28 (2010) 4384–4393.
[63] J. Alexander, J. Sidney, S. Southwood, J. Ruppert, C. Oseroff, A. Maewal, K. Snoke, H.M. Serra, R.T. Kubo, A. Sette, H.M. Grey, Development of high potency universal DR-restricted helper epitopes by modification of high affinity DR-blocking peptides, Immunity 1 (1994) 751–761.
[64] K. Jemon, V. Young, M. Wilson, S. McKee, V. Ward, M. Baird, S. Young, M. Hibma, An enhanced heterologous virus-like particle for human papillomavirus type 16 tumour immunotherapy, PLoS One 8 (2013), e66866. .
[65] A. Zeltins, J. West, F. Zabel, A. El Turabi, I. Balke, S. Haas, M. Maudrich, F. Storni, P. Engeroff, G.T. Jennings, A. Kotecha, D.I. Stuart, J. Foerster, M.F. Bachmann, Incorporation of Tetanus-epitope into virus-like particles achieves vaccine responses even in older recipients in models of psoriasis, Alzheimer's and cat allergy, Vaccines 2 (2017) 30.
[66] P.A. Kratz, B. Böttcher, M. Nassal, Native display of complete foreign protein domains on the surface of hepatitis B virus capsids, Proc. Natl. Acad. Sci. U. S. A. 96 (1999) 1915–1920.
[67] G.J. O'Brien, C.J. Bryant, C. Voogd, H.B. Greenberg, R.C. Gardner, A.R. Bellamy, Rotavirus VP6 Expressed by PVX Vectors in Nicotiana benthamiana Coats PVX Rods and also Assembles into Viruslike Particles, Virology 270 (2000) 444–453.
[68] R.M. Jones, J.A. Chichester, V. Mett, J. Jaje, S. Tottey, S. Manceva, L.J. Casta, S.K. Gibbs, K. Musiychuk, M. Shamloul, J. Norikane, V. Mett, S. Streatfield, M. van de Vegte-Bolmer, W. Roeffen, R.W. Sauerwein, V. Yusibov, A plant-produced Pfs25 VLP malaria vaccine candidate induces persistent transmission blocking antibodies against Plasmodium falciparum in immunized mice, PLoS One 8 (2013), e79538. .
[69] A. Walker, C. Skamel, M. Nassal, SplitCore: an exceptionally versatile viral nanoparticle for native whole protein display regardless of 3D structure, Sci. Rep. 1 (2011) 5.
[70] L.H. Lua, Y. Fan, C. Chang, N.K. Connors, A.P. Middelberg, Synthetic biology design to display an 18 kDa rotavirus large antigen on a modular virus-like particle, Vaccine 33 (2015) 5937–5944.
[71] L. Zhai, J. Peabody, Y. Pang, J. Schiller, B. Chackerian, E. Tumban, A novel candidate HPV vaccine: MS2 phage VLP displaying a tandem HPV L2 peptide offers similar protection in mice to Gardasil-9, Antivir. Res. 147 (2017) 123.
[72] D.F. Kelly, E.R. Moxon, A.J. Pollard, Haemophilus influenzae type b conjugate vaccines, Immunology 113 (2004) 163–174.
[73] E. Baslé, N. Joubert, M. Pucheault, Protein chemical modification on endogenous amino acids, Chem. Biol. 17 (2010) 213–227.
[74] A. Zeltins, Viral nanoparticles: principles of construction and characterization, in: Y.E. Khudyakov, P. Pumpens (Eds.), Viral Nanotechnology, CRC Press, Boca Raton 2016, pp. 93–119.
[75] N.F. Steinmetz, C.F. Cho, A. Ablack, J.D. Lewis, M. Manchester, Cowpea mosaic virus nanoparticles target surface vimentin on cancer cells, Nanomedicine (Lond). 6 (2011) 351–364.
[76] N.F. Steinmetz, M.E. Mertens, R.E. Taurog, J.E. Johnson, U. Commandeur, R. Fischer, M. Manchester, Potato virus X as a novel platform for potential biomedical applications, Nano Lett. 10 (2010) 305–312.
[77] A.A. McCormick, T.A. Corbo, S. Wykoff-Clary, K.E. Palmer, G.P. Pogue, Chemical conjugate TMV-peptide bivalent fusion vaccines improve cellular immunity and tumor protection, Bioconjug. Chem. 17 (2006) 1330–1338.
[78] N.L. Weineisen, C.A. Hommersom, J. Voskuhl, S. Sankaran, A.M. Depauw, N. Katsonis, P. Jonkheijm, J.J. Cornelissen, Photoresponsive, reversible immobilization of virus particles on supramolecular platforms, Chem. Commun. Camb. 53 (2017) 1896–1899.
[79] C. Koch, K. Wabbel, F.J. Eber, P. Krolla-Sidenstein, C. Azucena, H. Gliemann, S. Eiben, F. Geiger, C. Wege, Modified TMV particles as beneficial scaffolds to present sensor enzymes, Front. Plant Sci. 6 (2015) 1137.
[80] A. Thérien, M. Bédard, D. Carignan, G. Rioux, L. Gauthier-Landry, M.E. Laliberté-Gagné, M. Bolduc, P. Savard, D. Leclerc, A versatile papaya mosaic virus (PapMV) vaccine platform based on sortase-mediated antigen coupling, J. Nanobiotechnol. 15 (2017) 54.
[81] K.D. Brune, D.B. Leneghan, I.J. Brian, A.S. Ishizuka, M.F. Bachmann, S.J. Draper, S. Biswas, M. Howarth, Plug-and-display: decoration of virus-like particles via isopeptide bonds for modular immunization, Sci. Rep. 6 (2016), 19234. .
[82] M. Deschuyteneer, A. Elouahabi, D. Plainchamp, M. Plisnier, D. Soete, Y. Corazza, L. Lockman, S.M. Giannini, M. Deschamps, Molecular and structural characterization of the L1 virus-like particles that are used as vaccine antigens in Cervarix™, the AS04-adjuvanted HPV-16 and -18 cervical cancer vaccine, Hum. Vaccine 6 (2010) 407–419.
[83] . S.W. Li, Q. Zhao, T. Wu, S. Chen, J. Zhang, N.S. Xia, The development of a recombinant hepatitis E vaccine HEV 239, Hum.Vaccine Immunother. 11 (2015) 908–914.
[84] X. Lu, J.R. Thompson, K.L. Perry, Encapsidation of DNA, a protein and a fluorophore into virus-like particles by the capsid protein of cucumber mosaic virus, J. Gen. Virol. 93 (2012) 1120–1126.
[85] Y. Xu, J. Ye, H. Liu, E. Cheng, Y. Yang, W. Wang, M. Zhao, D. Zhou, D. Liu, R. Fang, DNA-templated CMV viral capsid proteins assemble into nanotubes, Chem. Commun. (Camb). 7 (2008) 49–51.
[86] A. Jegerlehner, F. Zabel, A. Langer, K. Dietmeier, G.T. Jennings, P. Saudan, M.F. Bachmann, Bacterially produced recombinant influenza vaccines based on virus-like particles, PLoS One 8 (2013), e78947. .
[87] J. Jobsri, A. Allen, D. Rajagopal, M. Shipton, K. Kanyuka, G.P. Lomonossoff, C. Ottensmeier, S.S. Diebold, F.K. Stevenson, N. Savelyeva, Plant virus particles carrying tumour antigen activate TLR7 and Induce high levels of protective antibody, PLoS One 10 (2015), e0118096. .
[88] F.R. Brennan, T. Bellaby, S.M. Helliwell, T.D. Jones, S. Kamstrup, K. Dalsgaard, J.I. Flock, W.D. Hamilton, Chimeric plant virus particles administered nasally or orally induce systemic and mucosal immune responses in mice, J. Virol. 73 (1999) 930–938.
[89] M.E. Lebel, J.F. Daudelin, K. Chartrand, E. Tarrab, U. Kalinke, P. Savard, N. Labrecque, D. Leclerc, A. Lamarre, Nanoparticle adjuvant sensing by TLR7 enhances CD8+ T cell-mediated protection from Listeria monocytogenes infection, J. Immunol. 192 (2014) 1071–1078.
[90] C.A. Manuel-Cabrera, A.A. Vallejo-Cardona, E. Padilla-Camberos, R. Hernández-Gutiérrez, S.E. Herrera-Rodríguez, A. Gutiérrez-Ortega, Self-assembly of hexahistidine-tagged tobacco etch virus capsid protein into microfilaments that induce IgG2-specific response against a soluble porcine reproductive and respiratory syndrome virus chimeric protein, Virol. J. 13 (2016) 196.
[91] E.A. Trifonova, V.A. Zenin, N.A. Nikitin, M.S. Yurkova, E.M. Ryabchevskaya, E.V. Putlyaev, E.K. Donchenko, O.A. Kondakova, A.N. Fedorov, J.G. Atabekov, O.V. Karpova, Study of rubella candidate vaccine based on a structurally modified plant virus, Antivir. Res. 144 (2017) 27–33.
[92] P.S. Satheshkumar, G.L. Lokesh, M.R. Murthy, H.S. Savithri, The role of arginine-rich motif and beta-annulus in the assembly and stability of Sesbania mosaic virus capsids, J. Mol. Biol. 353 (2005) 447–458.
[93] K. Matsuura, K. Watanabe, T. Matsuzaki, K. Sakurai, N. Kimizuka, Self-assembled synthetic viral capsids from a 24-mer viral peptide fragment, Angew. Chem. Int. Ed. 49 (2010) 9662–9665.
[94] K. Matsuura, T. Nakamura, K. Watanabe, T. Noguchi, K. Minamihata, N. Kamiya, N. Kimizuka, Self-assembly of Ni-NTA-modified β-annulus peptides into artificial viral capsids and encapsulation of His-tagged proteins, Org. Biomol. Chem. 14 (2016) 7869–7874.
[95] M.L. Smith, J.A. Lindbo, S. Dillard-Telm, P.M. Brosio, A.B. Lasnik, A.A. McCormick, L.V. Nguyen, K.E. Palmer, Modified tobacco mosaic virus particles as scaffolds for display of protein antigens for vaccine applications, Virology 348 (2006) 475–788.
[96] S. Pejawar-Gaddy, Y. Rajawat, Z. Hilioti, J. Xue, D.F. Gaddy, O.J. Finn, R.P. Viscidi, I. Bossis, Generation of a tumor vaccine candidate based on conjugation of a MUC1 peptide to polyionic papillomavirus virus-like particles, Cancer Immunol. Immunother. 59 (2010) 1685–1696.
[97] I. Kalnciema, I. Balke, D. Skrastina, V. Ose, A. Zeltins, Potato virus M-like nanoparticles: construction and characterization, Mol. Biotechnol. 57 (2015) 982–992.
[98] A. Abraham, U. Natraj, A.A. Karande, A. Gulati, M.R. Murthy, S. Murugesan, P. Mukunda, H.S. Savithri, Intracellular delivery of antibodies by chimeric Sesbania mosaic virus (SeMV) virus like particles, Sci. Rep. 6 (2016), 21803. .
[99] M.B. van Eldijk, J.C. Wang, I.J. Minten, C. Li, A. Zlotnick, R.J. Nolte, J.J. Cornelissen, J.C. van Hest, Designing two self-assembly mechanisms into one viral capsid protein, J. Am. Chem. Soc. 134 (2012) 18506–18509.
[100] I.J. Minten, L.J. Hendriks, R.J. Nolte, J.J. Cornelissen, Controlled encapsulation of multiple proteins in virus capsids, J. Am. Chem. Soc. 131 (2009) 17771–17773.
[101] C. Savard, M.E. Laliberté-Gagné, C. Babin, M. Bolduc, A. Guérin, K. Drouin, M.A. Forget, N. Majeau, R. Lapointe, D. Leclerc, Improvement of the PapMV nanoparticle adjuvant property through an increased of its avidity for the antigen [influenza NP], Vaccine 30 (2012) 2535–2542.
[102] M.O. Mohsen, A.C. Gomes, G. Cabral-Miranda, C.C. Krueger, F.M. Leoratti, J.V. Stein, M.F. Bachmann, Delivering adjuvants and antigens in separate nanoparticles eliminates the need of physical linkage for effective vaccination, J. Control. Release 251 (2017) 92–100.

[103] J.P. Phelps, N. Dang, L. Rasochova, Inactivation and purification of cowpea mosaic virus-like particles displaying peptide antigens from bacillus anthracis, J. Virol. Methods 141 (2007) 146–153.
[104] W.O. Dawson, D.L. Beck, D.A. Knorr, G.L. Grantham, cDNA cloning of the complete genome of tobacco mosaic virus and production of infectious transcripts, Proc. Natl. Acad.Sci. U.S.A. 83 (1986) 1832–1836.
[105] K.E. Palmer, A. Benko, S.A. Doucette, T.I. Cameron, T. Foster, K.M. Hanley, A.A. McCormick, M. McCulloch, G.P. Pogue, M.L. Smith, N.D. Christensen, Protection of rabbits against cutaneous papillomavirus infection using recombinant tobacco mosaic virus containing L2 capsid epitopes, Vaccine 24 (2006) 5516–5525.
[106] K. Musiychuk, N. Stephenson, H. Bi, C.E. Farrance, G. Orozovic, M. Brodelius, P. Brodelius, A. Horsey, N. Ugulava, A.M. Shamloul, V. Mett, S. Rabindran, S.J. Streatfield, V. Yusibov, A launch vector for the production of vaccine antigens in plants, Influenza Other Respir. Viruses 1 (2007) 19–25.
[107] Y. Gleba, V. Klimyuk, S. Marillonnet, Magnifection: a new platform for expressing recombinant vaccines in plants, Vaccine 23 (2005) 2042–2048.
[108] K. Hefferon, Plant virus expression vectors: a powerhouse for global health, Biomedicine 5 (2017) 44.
[109] H. Peyret, G.P. Lomonossoff, When plant virology met Agrobacterium: the rise of the deconstructed clones, Plant Biotechnol. J. 13 (2015) 1121–1135.
[110] X. Zhao, J.M. Fox, N.H. Olson, T.S. Baker, M.J. Young, In vitro assembly of cowpea chlorotic mottle virus from coat protein expressed in *Escherichia coli* and in vitro-transcribed viral cDNA, Virology 207 (1995) 486–494.
[111] J.P. Phelps, P. Dao, H. Jin, L. Rasochova, Expression and self-assembly of cowpea chlorotic mottle virus-like particles in Pseudomonas fluorescens, J. Biotechnol. 128 (2007) 290–296.
[112] S. Brumfield, D. Willits, L. Tang, J.E. Johnson, T. Douglas, M. Young, Heterologous expression of the modified coat protein of Cowpea chlorotic mottle bromovirus results in the assembly of protein cages with altered architectures and function, J. Gen. Virol. 85 (2004) 1049–1053.
[113] K. Saunders, F. Sainsbury, G.P. Lomonossoff, Efficient generation of cowpea mosaic virus empty virus-like particles by the proteolytic processing of precursors in insect cells and plants, Virology 393 (2009) 329–337.
[114] S. Kumar, W. Ochoa, P. Singh, C. Hsu, A. Schneemann, M. Manchester, M. Olson, V. Reddy, Tomato bushy stunt virus (TBSV), a versatile platform for polyvalent display of antigenic epitopes and vaccine design, Virology 388 (2009) 185–190.
[115] J.M. Hammond, K.W. Sproat, T.G. Wise, A.D. Hyatt, M.N. Jagadish, B.E.H. Coupar, Expression of the potyvirus coat protein mediated by recombinant vaccinia virus and assembly of potyvirus-like particles in mammalian cells, Arch. Virol. 143 (1998) 1433–1439.
[116] N. Kushnir, S.J. Streatfield, V. Yusibov, Virus-like particles as a highly efficient vaccine platform: Diversity of targets and production systems and advances in clinical development, Vaccine 31 (2012) 58–83.
[117] H.J. Kim, H.J. Kim, Yeast as an expression system for producing virus-like particles: what factors do we need to consider? Lett. Appl. Microbiol. 64 (2017) 111–123.
[118] G. Lanza, Building today's platform company, Nat. Biotechnol. 27 (2009) 693–695.
[119] H.K. Charlton Hume, L.H.L. Lua, Platform technologies for modern vaccine manufacturing, Vaccine 35 ((2017) 4480–4485.
[120] V. Yusibov, D.C. Hooper, S.V. Spitsin, N. Fleysh, R.B. Kean, T. Mikheeva, D. Deka, A. Karasev, S. Cox, J. Randall, H. Koprowski, Expression in plants and immunogenicity of plant virus-based experimental rabies vaccine, Vaccine 20 (2002) 3155–3164.
[121] Fraunhofer Center for Molecular Biotechnology, Safety and Immunogenicity of Plant-Derived Pfs25 VLP-FhCMB Malaria Transmission Blocking Vaccine in Healthy Adults, https://clinicaltrials.gov/ct2/show/NCT02013687 (accessed December 17, 2017).
[122] Malaria Vaccine Iniciative, Pfs25 VLP and Pfs230 VLP, http://www.malariavaccine.org/projects/vaccine-projects/pfs25-vlp-and-pfs230-vlp-0, Accessed date: 17 December 2017.
[123] Folia Biotech Inc, Safety and Reactogenicity of a PAL Combined With Seasonal Flu Vaccine in Healthy Adults, https://clinicaltrials.gov/ct2/show/record/NCT02188810, Accessed date: 17 December 2017.
[124] A. Gellert, K. Salanki, K. Tombacz, T. Tuboly, E. Balazs, A cucumber mosaic virus based expression system for the production of porcine circovirus specific vaccines, PLoS One 7 (2012), e52688. .
[125] J.P. Langeveld, F.R. Brennan, J.L. Martínez-Torrecuadrada, T.D. Jones, R.S. Boshuizen, C. Vela, J.I. Casal, S. Kamstrup, K. Dalsgaard, R.H. Meloen, M.M. Bendig, W.D. Hamilton, Inactivated recombinant plant virus protects dogs from a lethal challenge with canine parvovirus, Vaccine 19 (2001) 3661–3670.
[126] C.D. Yang, J.T. Liao, C.Y. Lai, M.H. Jong, C.M. Liang, Y.L. Lin, N.S. Lin, Y.H. Hsu, S.M. Liang, Induction of protective immunity in swine by recombinant bamboo mosaic virus expressing foot-and-mouth disease virus epitopes, BMC Biotechnol. 7 (2007) 62.
[127] M.F. Bachmann, A. Zeltins, G. Kalnins, I. Balke, N. Fischer, A. Rostaher, K. Tars, C. Favrot, Vaccination against IL-31 for the treatment of atopic dermatitis in dogs, J. Allergy Clin. Immunol. 142 (2018) 279–281.
[128] A. Fettelschoss-Gabriel, V. Fettelschoss, F. Thoms, C. Giese, M. Daniel, F. Olomski, J. Kamarachev, K. Birkmann, M. Bühler, M. Kummer, A. Zeltins, E. Marti, T.M. Kündig, M.F. Bachmann, Treating insect-bite hypersensitivity in horses with active vaccination against IL-5, J. Allergy Clin. Immunol. (2018)https://doi.org/10.1016/j.jaci.2018.01.041 in press.
[129] T. Zhang, M. Breitbart, W.H. Lee, J.Q. Run, C.L. Wei, S.W.T. Soh, M.L. Hibberd, E.T. Liu, F. Rohwer, Y. Ruan, RNA viral community in human feces: prevalence of plant pathogenic viruses, PLoS Biol. (4) (2006) e3.
[130] P. Colson, H. Richet, C. Desnues, F. Balique, V. Moal, J.J. Grob, P. Berbis, H. Lecoq, J.-R. Harlé, Y. Berland, D. Raoult, Pepper mild mottle virus, a plant virus associated with specific immune responses, fever, abdominal pains, and pruritus in humans, PLoS One 5 (2010), e10041. .
[131] H.S. Mason, D.M. Lam, C.J. Arntzen, Expression of hepatitis B surface antigen in transgenic plants, Proc. Natl. Acad. Sci. U. S. A. 89 (1992) 11745–11749.
[132] G.P. Pogue, J.A. Lindbo, S.J. Garger, W.P. Fitzmaurice, Making an ally from an enemy: plant virology and the new agriculture, Annu. Rev. Phytopathol. 40 (2002) 45–74.
[133] C.S. Rae, I.W. Khor, Q. Wang, G. Destito, M.J. Gonzalez, P. Singh, D.M. Thomas, M.N. Estrada, E. Powell, M.G. Finn, M. Manchester, Systemic trafficking of plant virus nanoparticles in mice via the oral route, Virology 343 (2005) 224–235.
[134] C.R. Kaiser, M.L. Flenniken, E. Gillitzer, A.L. Harmsen, A.G. Harmsen, M.A. Jutila, T. Douglas, M.J. Young, Biodistribution studies of protein cage nanoparticles demonstrate broad tissue distribution and rapid clearance in vivo, Int. J. Nanomedicine 2 (2007) 715–733.
[135] P. Singh, D. Prasuhn, R.M. Yeh, G. Destito, C.S. Rae, K. Osborn, M.G. Finn, M. Manchester, Bio-distribution, toxicity and pathology of cowpea mosaic virus nanoparticles in vivo, J. Control. Release 120 (2007) 41–50.
[136] M.A. Bruckman, L.N. Randolph, A. Van Meter, S. Hern, A.J. Shoffstall, R.E. Taurog, N.F. Steinmetz, Biodistribution, pharmacokinetics, and blood compatibility of native and PEGylated tobacco mosaic virus nano-rods and -spheres in mice, Virology 449 (2014) 163–173.
[137] J.F.C. Steele, H. Peyret, K. Saunders, R. Castells-Graells, J. Marsian, Y. Meshcheriakova, G.P. Lomonossoff, Synthetic plant virology for nanobiotechnology and nanomedicine, WIREs Nanomed. Nanobiotechnol. 9 (2017), e1447. .
[138] J.V. Sarma, P.A. Ward, The complement system, Cell Tissue Res. 343 (2011) 227–235.
[139] A.S. Pitek, A.M. Wen, S. Shukla, N.F. Steinmetz, The protein corona of plant virus nanoparticles influences their dispersion properties, cellular interactions and in vivo fates, Small 12 (2016) 1758–1769.
[140] M.E. Lebel, M.P. Langlois, J.F. Daudelin, E. Tarrab, P. Savard, D. Leclerc, A. Lamarre, Complement component 3 regulates IFN-α production by plasmacytoid dendritic cells following TLR7 activation by a plant virus-like nanoparticle, J. Immunol. 198 (2017) 292–299.
[141] R.P. Friedland, Mechanisms of molecular mimicry involving the microbiota in neurodegeneration, J. Alzheimers Dis. 45 (2015) 349–362.
[142] L. Liljeroos, E. Malito, I. Ferlenghi, M.J. Bottomley, Structural and computational biology in the design of immunogenic vaccine antigens, J Immunol Res 2015 (2015) 156241.
[143] K. Uhde-Holzem, V. Schlosser, S. Viazov, R. Fischer, U. Commandeur, Immunogenic properties of chimeric potato virus X particles displaying the hepatitis C virus hypervariable region I peptide R9, J. Virol. Methods 166 (2010) 12–20.
[144] M. Nuzzaci, G. Piazzolla, A. Vitti, M. Lapelosa, C. Tortorella, I. Stella, A. Natilla, S. Antonaci, P. Piazzolla, Cucumber mosaic virus as a presentation system for a double hepatitis C virus-derived epitope, Arch. Virol. 152 (2007) 915–928.
[145] D.B. Leneghan, K. Miura, I.J. Taylor, Y. Li, J. Jin, K.D. Brune, M.F. Bachmann, M. Howarth, C.A. Long, S. Biswas, Nanoassembly routes stimulate conflicting antibody quantity and quality for transmission-blocking malaria vaccines, Sci. Rep. 7 (2017) 3811.
[146] A.C. Gomes, A. Flace, P. Saudan, F. Zabel, G. Cabral-Miranda, A.E. Turabi, V. Manolova, M.F. Bachmann, Adjusted particle size eliminates the need of linkage of antigen and adjuvants for appropriated T cell responses in virus-like particle-based vaccines, Front. Immunol. 8 (2017) 226.
[147] A.S. Rose, P.W. Hildebrand, NGL Viewer: a web application for molecular visualization, Nucl. Acids. Res. 43 (2015) W576–W579.
[148] T. Lin, Z. Chen, R. Usha, C.V. Stauffacher, J.B. Dai, T. Schmidt, J.E. Johnson, The refined crystal structure of cowpea mosaic virus at 2.8 a resolution, Virology 265 (1999) 20–34.
[149] P. Ge, Z.H. Zhou, Hydrogen-bonding networks and RNA bases revealed by cryo electron microscopy suggest a triggering mechanism for calcium switches, Proc. Natl. Acad. Sci.USA 108 (2011) 9637–9642.
[150] J.M. Antos, M.C. Truttmann, H.L. Ploegh, Recent advances in sortase-catalyzed ligation methodology, Curr. Opinion Struct. Biol. 38 (2016) 111–118.
[151] S.C. Reddington, M. Howarth, Secrets of a covalent interaction for biomaterials and biotechnology: SpyTag and SpyCatcher, Curr. Opinion Chem. Biol. 29 (2015) 94–99.