Virus-like particles: Passport to immune recognition

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Abstract

Virus-like particles (VLPs) are formed by the self-assembly of envelope and/or capsid proteins from many viruses. In many cases such VLPs have structural characteristics and antigenicity similar to the parental virus, and some have already proven successful as vaccines against the cognate virus infection. The structural components of some VLPs have also proven amenable to the insertion or fusion of foreign antigenic sequences, allowing the production of chimeric VLPs exposing the foreign antigen on their surface. Other VLPs have been used as carriers for foreign antigens, including non-protein antigens, via chemical conjugation. This review outlines some of the advantages, disadvantages, and technical considerations for the use of a wide range of VLP systems in vaccine development.
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1. Introduction

The structural proteins of many viruses have the ability to assemble into repeated arrays, or virus-like particles (VLPs), following recombinant DNA expression in a variety of culture systems. Such VLPs fall in the general size range of viruses (22–150 nm), with their exact size and morphology depending on the particular viral proteins incorporated, but VLPs are non-infectious because they assemble without incorporating genetic material. VLPs offer a promising approach to the production of vaccines against many diseases, because their repetitive, high density display of epitopes is often effective in eliciting strong immune responses. This is further enhanced by the particulate nature of VLPs, especially in the size range of around 40 nm that appears to be optimal for uptake of nanoparticles by dendritic cells [1].

2. General characteristics of VLPs

In some cases, VLPs are similar to naturally occurring subviral particles (SVPs). For example, expression of the small envelope protein of hepatitis B virus (HBV) in yeast or mammalian cells leads to the formation of 22 nm VLPs that are essentially identical to the SVPs that are a natural product of HBV infection, found in patient blood at levels far greater than the virion itself. Notably, these plasma-derived SVPs provided the first-generation HBV vaccines. Similarly, expression of the L1 protein of human papillomavirus leads to the assembly of VLPs that are somewhat similar to the "empty" virus particles formed during papillomavirus replication, although the natural empty particles also contain the L2 protein. Recombinant human papillomavirus vaccines, based on the L1 protein of HPV, are expected to be licensed in the near future. Further examples of VLPs as vaccine candidates are shown in Table 1.

In other cases, VLPs can be exploited as "platforms" for the presentation of foreign epitopes and/or targeting molecules on chimeric VLPs. This can be achieved through modification of the VLP gene sequence(s), such that fusion proteins of VLP proteins and foreign vaccine proteins are assembled into VLPs during *de novo* synthesis. Fusion of peptide sequences with the core gene (HBcAg) of hepatitis B virus provided an early example of this approach. Alternatively, foreign vaccine proteins may be chemically conjugated to pre-formed VLPs. As one example, this approach has been used in the production of HBcAg VLPs contain-

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Table 1
Examples of VLPs used for vaccines and vaccine development

Virus	Particle composition	Type/expression system	Size	Vaccine status	References
HBV	Small envelope protein (HBsAg)	rec VLP (yeast) (Recombivax-HB; Engerix-B)	22 nm	Licensed	[46,47]
	Small envelope protein (HBsAg)	rec VLP (potato)	17 nm	Preclinical	[48]
	PreS1+2 and HBsAg	rec VLP (CHO cells) (Sci-B-Vac; BioHepB)	22 nm	Licensed	[9,10,12,13]
	HBsAg	Native SVP (plasma)	22 nm	Licensed (developing world)	[49]
HPV	L1, major capsid protein	recVLP (mammalian cells; baculovirus; yeast) Gardasil, Cervarix	40–50 nm	Licensed	[50–53]
HEV	Truncated major capsid protein (ORF2)	rec VLP (baculovirus)	23.7 nm		[54–56] [57] (review)
Influenza	HA, NA, matrix	recVLP (baculovirus)	80–120 nm	Preclinical	[14–16]
HCV	Core, E1, E2	recVLP (baculovirus)	40–60 nm	Preclinical	[58–61]
Poliovirus	Capsid (VP0,1,3)	recVLP (baculovirus)	27 nm	None	[3]
HIV	Pr55gag, envelope	recVLP (baculovirus; mammalian cells; yeast)	100–120 nm	Preclinical	[62,63] [64,65] (review) [18] (review)
Ebola virus; Marburg virus	Glycoprotein (GP) and matrix (VP40)	recVLP (mammalian cells)	Filovirus-like particle	Preclinical	[66–68]
Norwalk virus	capsid	rec VLP (baculovirus; transgenic potatoes)	38 nm	Phase1	[69,44,70]
Rotavirus	VP2,VP6,VP7	recVLP (baculovirus)	70–75 nm	Preclinical	[5,71,72]
SARS coronavirus	S, E and M	rec VLP (baculovirus)	100 nm	Preclinical	[73]

Abbreviations: HBV, hepatitis B virus; HPV, human papilloma virus; HEV, hepatitis E virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus; SARS, severe acute respiratory syndrome.

ing the extracellular domain of the M2 ion channel protein of influenza A virus. The chemical conjugation approach also allows VLPs to be exploited for non-protein antigens, such as nicotine [2]. Further examples of chimeric VLPs are shown in Table 2.

Different viruses provide a variety of building blocks for the production of VLPs. For non-enveloped viruses (such as HPV), or core particles of enveloped viruses (such as HBcAg), one or more chimeric capsid proteins are expressed for self-assembly. Multiple capsid proteins may be assembled either from expression and subsequent processing of a precursor protein [3,4] or by co-expression of the capsid proteins from bicistronic or multicistronic vectors in the same cell [5]. For enveloped viruses, VLPs are assembled from envelope proteins with or without the matrix/capsid proteins that form the authentic viral particle. In either case, the envelope proteins bud from the usual cellular compartments (ER, plasma membrane or lipid rafts associated with the plasma membrane) and thus contain the cellular lipids that make up the viral lipoprotein envelope. In some instances the VLPs may also include host cell proteins, for example, the lipid raft associated ganglioside M1 in Ebola and Marburg VLPs, [6] and CD55, CD59 and CD46 in HIV, which help evade complement lysis [7].

3. VLPs and the induction of humoral immune responses

VLPs are commonly more immunogenic than subunit or recombinant protein immunogens, and are able to stimulate both the humoral and cellular arms of the immune system. VLPs provide the spatial structure for display of conformational epitopes and in doing so are most likely to mimic the native virus structure, thereby enhancing the production of neutralizing antibodies. This may be especially true for surface proteins of parasites and enveloped viruses, where enveloped VLPs allow the vaccine proteins to be presented in their natural state as membrane-bound proteins, rather than as the soluble ectodomains alone. VLPs may therefore offer a safe and effective approach for the induction of antibody to surface proteins where soluble forms of the proteins have failed to be effective.

Some VLPs also have the capacity to present multiple vaccine proteins to the immune system. Despite the success of the HBV vaccine, improvements to its efficacy have been sought by inclusion of the large and middle envelope proteins to the HBsAg particle, thereby mimicking the authentic virion envelope which contains these three proteins [8–12]. HBV vaccines which include these additional envelope proteins have to varying extents elicited antibody responses to these proteins, but more specifically they

Table 2
Examples of chimeric VLPs as platforms for vaccine development

VLP platforms	Chimeric antigen(s)	Expression system	References
HBc	GFP; malaria epitopes; HBV preS1; immunodominant epitopes of numerous viral pathogens, including bacterial and protozoan epitopes (reviewed in [33])	*E. coli*	[33,74–78, 41]
WHBc	Various model epitopes	*E. coli*	[79]
HBs	HCV HVR1; plant signal peptides; Dengue virus envelope protein; HIV gp41 2F5 epitope	Mammalian cells; tobacco plants; yeast	[80] [81] [82–85]
HPV	SHIV (HIV tat, rev; SIV gag); HPV E6/E7	Baculovirus	[32,86]
BPV	CTL epitopes of HPV and HIV	Baculovirus	[87]
Yeast Ty	HIV V3 loop; HIV p24; malaria epitopes	Yeast	[88–90]
HIV; SHIV gag	Various HIV env epitopes	Baculovirus; mammalian cells	[65] (review) [18] (review)
DHBV	HCV E2; various antigens	Yeast	Grgacic and Anderson, unpublished
HEV	HEV B cell epitope	Baculovirus	[91]
Phage Qbeta	Nicotine	*E. coli*	[2]

Abbreviations: HBc, hepatitis B virus core; WHBc, woodchuck hepatitis B virus core; HBs, hepatitis B virus S antigen; HPV, human papillomavirus; BPV, bovine papillomavirus; HIV, human immunodeficiency virus; SHIV, simian immunodeficiency virus HIV chimera; DHBV, duck hepatitis B virus; HEV, hepatitis E virus.

provide additional T cell epitopes which promote an earlier anti-HBsAg response and improve the seroprotection rate by overcoming non-responsiveness to the HBsAg vaccine [11–13].

Influenza A virus presents an example where recombinant VLPs have been developed that closely mimic the native virus particle [14–16]. Influenza VLPs have a similar size to the native influenza virus particle (around 120 nm), and provide a useful alternative to egg-derived or cell-culture derived vaccines based on inactivated virus. However, the large size of the influenza VLPs may not be optimal for immunogenicity.

HIV presents another example where substantial efforts have been made to recreate the virion envelope in a form that permits the efficient induction of broadly neutralising antibodies. This has included the production of synthetic proteoliposomes on solid microspherical beads in order to mimic the lipid membrane environment for presentation of HIV gp160 [17], as well as VLPs mimicking the intact HIV particle, containing the envelope proteins associated with the viral gag protein core (see [18] for a more extensive review of HIV VLP vaccines). This has evolved with increasing knowledge of envelope structure through X-ray crystallography and mutational analysis of key envelope domains associated with viral entry, coupled with the understanding that conserved regions, unlikely to undergo antigenic variation through immune pressure, are essential targets for eliciting a broadly neutralising antibody response (reviewed in [18,19]). Recently, research has focused on presentation of highly conserved, neutralizing epitopes of the HIV-1 gp41 envelope protein in a prefusion conformation, which is only briefly exposed during viral entry (reviewed by McGaughey [20]). These specific goals for vaccine development are likely to require experimentation with the wide variety of VLPs and expression systems available and the use of functional assays, such as receptor binding, reactivity to conformation-specific antibodies, and most importantly the induction of broadly cross-neutralizing antibodies, as a means to confirm the appropriate envelope protein folding for vaccine efficacy.

4. VLPs, dendritic cells and the innate and adaptive cellular immune responses

In some cases, VLPs do not appear to require the use of adjuvants to achieve potent immune stimulation. The self-adjuvanting effects of such VLPs are inherent in their tendency to be a suitable size for uptake by dendritic cells (DCs) for processing and presentation by MHC class II and for directly promoting DC maturation and migration, essential for stimulation of the innate immune response [1,21]. Exogenous VLPs can also be taken up and processed via the MHC class 1 pathway (cross-presentation) for activation of CD8^{+} T-cells, which are essential for the clearance of intracellular pathogens such as viruses. The ability of VLPs to target DCs is an important advantage of VLP vaccines, as targeting of this cell type is now understood to be essential for activating the innate and adaptive immune responses. Some VLPs that resemble infectious viruses and retain their receptor binding regions are able to target and enter cells via their normal receptor and are taken up by antigen presenting cells as exogenous antigens for class I presentation. Receptor-mediated uptake has been shown to occur via sialic acid interaction with the haemagglutinin/

neuraminidase of paramyxoviruses [22], CD4 interaction with HIV-1 [23] and while binding to cells by HCV is envelope-specific, the receptor involved is still unclear [24]. Stimulation of DCs to produce cytokines, such as interferon- α/β, does not require replication of the virus but rather an intact envelope of either an inactivated virus [25] or that of a VLP, as shown for Ebola and Marburg viruses [26], or an intact non-enveloped VLP, such as HPV [27,28]. In this context VLPs may have advantages over the cognate live viruses for immune activation, because several viruses that replicate in DCs are known to block activation and maturation of the cell through expression of particular viral proteins [29–31], and even inactivated Ebola and Marburg viruses still interfere with DC activation, suggesting a viral protein present on the virus and not the VLP is responsible [26].

5. Tailoring VLPs for vaccine development

VLPs have been produced from the capsid or envelope components of a wide variety of viruses for the purpose of studying viral assembly and for the development of vaccines. While HBV and HPV VLPs have made successful vaccines, pathogens that directly affect immune cells and those that successfully evade the immune system, such as HIV-1 and hepatitis C virus have proven to be more challenging. It is clear that many individuals are exposed to large amounts of these authentic viral particle for years during chronic infection without developing a fully effective immune response, which suggests that VLPs may also prove to be ineffective even when they closely mimic the native viral structure. Some candidates may thus require adaptations in VLP design (such as particle size, envelope structure), targeting in the host (DCs, mucosal surfaces) and route of administration to achieve the desired immune response.

Chimeric VLPs provide a means for the incorporation of heterologous antigens into VLPs, including antigens that are unable to self-assemble in a particulate form (such as CTL epitopes and fragments of envelope proteins), and antigens from viruses where the intact virus particle may not have optimal immunogenicity (such as HIV and HCV). Chimeric VLPs may consist of a homologous VLP platform assembled with the fusion of epitopes from other or multiple strains of the virus or additional epitopes to the core VLP structure, such as fusion of the E6/E7 non-structural proteins to the L1 capsid protein of HPV [32]. Alternatively, chimeric VLPs may consist of a VLP platform for presentation of polyproteins or epitopes of a totally unrelated virus or pathogen (e.g. HBV core VLPs with malaria or human rhinovirus epitopes [33] or influenza M2 [34–37]). A major limitation to some VLP platforms is the small size of foreign epitopes that can be accommodated within particles, which can preclude the presentation of large antigens such as HIV envelope or influenza haemagglutinin proteins.

Tailoring of VLPs may also be influenced by whether the vaccine is to act as a prophylactic or therapeutic vaccine, with the latter thought to require the inclusion of specific T cell epitopes for CTL based clearance (HBV preS1/2 vaccine) or as in the case of HPV the oncogenic E6/E7 proteins [38]. The effectiveness of VLPs as therapeutic vaccines may be aided by the addition of adjuvants such as CpGs which stimulate DCs via Toll-like receptor 9, with precise co-localisation and co-effectiveness of the VLP/adjuvant made possible by techniques such as the packaging of CpGs into the VLPs. Vaccination with chimeric hepatitis B core VLPs with a CTL epitope of the lymphocytic choriomeningitis virus glycoprotein packaged with CpGs was able to induce high frequencies of peptide specific CD8+ T cells and cure mice of fibrosarcoma tumours with a single dose [39]. Moreover, the effectiveness of VLPs as T cell-based vaccines can be increased when administered as part of a DNA prime-VLP boost protocol [40]. Other ways of utilising VLPs as a delivery platform is through chemical cross-linking of peptide epitopes to reactive sites on the platform. This has been done by coupling peptides containing a free cysteine residue with lysines situated in the immunodominant exposed region on HBV core particles [41].

Chimeric VLPs offer enormous potential in selective, multi-epitope presentation but their success will be dependent on a clear understanding of the correlates of immune clearance or protection, including the selection of the most relevant epitopes for vaccine efficacy. This knowledge is lacking for many diseases. Of the numerous chimeric VLPs (Table 2), the "second generation" recombinant HBV vaccines, Sci-B-Vac and BioHepB, have shown the most progress in clinical trials. These VLPs include the two additional envelope proteins (L and M) found in the 42 nm virion but these proteins are incorporated at much lower levels, and the particles are only 22 nm in size [12]. Notably, these vaccines retain the major component of the first-generation recombinant HBsAg vaccines as well as the L and M proteins.

6. Manufacturing considerations for VLPs

There are many expression systems for the production of VLPs and these include: (1) various mammalian cell lines, either transiently or stably transfected or transduced with viral expression vectors; (2) the baculovirus/insect cell system; (3) various species of yeast including *Saccharomyces cerevisiae* and *Pichia pastoris*; and (4) *Escherichia coli* and other bacteria. Oral vaccine initiatives have also produced HBV and Norwalk virus VLPs from various plants, including tobacco and lettuce leaves as well as potato [42–44]. Ease of expression, ability to scale-up and cost of production have made yeast a popular choice, however considerations such as appropriate protein glycosylation and correct folding and assembly as well as codon optimisation may dictate alternative production systems. *E. coli* does not allow for glycosylation, while yeast and baculovirus are limited to high mannose glycoprotein modification, and this is sometimes inconsistent. Baculovirus-driven expression of influenza VLPs has provided the novel challenge of separating

the progeny baculovirus vector particles from the influenza VLPs, with both having a similar size range of 80–120 nm [14]. Mammalian cell culture systems are favoured for appropriate modifications and authentic assembly, but are a less controllable system and more costly for production. Retroviruses in particular also tend to include unwanted host cell membrane proteins in their envelope during assembly. Future directions in manufacturing may include approaches such as *in vitro* chemical self-assembly of VLPs based, in the first instance, on capsid components [45].

7. Conclusions

A wide variety of VLPs have shown promising results in small animal models, and may offer great potential for the development of vaccines against many difficult target diseases. However, manufacturing considerations are likely to limit the practical utility of many VLP approaches, while the small size of vaccine antigens that can be incorporated into some VLPs may also prove to be a significant barrier to vaccine efficacy. The anticipated licensure of the human papillomavirus VLP vaccines will undoubtedly provide further stimulus in this field.

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