CHAPTER 11

Virus-Like Particles as a Vaccine Delivery System:
Myths and Facts

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Abstract

Vaccines against viral disease have traditionally relied on attenuated virus strains or inactivation of infectious virus. Subunit vaccines based on viral proteins expressed in heterologous systems have been effective for some pathogens, but have often suffered from poor immunogenicity due to incorrect protein folding or modification. In this chapter we focus on a specific class of viral subunit vaccine that mimics the overall structure of virus particles and thus preserves the native antigenic conformation of the immunogenic proteins. These virus-like particles (VLPs) have been produced for a wide range of taxonomically and structurally distinct viruses, and have unique advantages in terms of safety and immunogenicity over previous approaches. With new VLP vaccines for papillomavirus beginning to reach the market place we argue that this technology has now 'come-of-age' and must be considered a viable vaccine strategy.

Introduction

There are many infectious viruses that remain major threats to public health (see Table 1). Where an effective vaccine exists, vaccination is usually the most cost-effective long-term protection against disease and spread for most viruses. The principle of vaccination is to generate sufficient immunity to protect from infectious disease. Thus the vaccine stimulates the body's natural defenses against disease through use of a benign 'decoy' that mimics the virulent pathogen. The more similar a vaccine is to the natural disease, the better the immune response to the pathogen on subsequent exposure. In general, resistance to virus infection depends on the development of an immune response to antigens present on the surface of virions or virus-infected cells. Therefore identification of protective antigens is the first step in the development of effective viral vaccines.

Currently many successful viral vaccines have been developed and are in use. These vaccines are predominantly based on live attenuated or inactivated viruses. The live attenuated vaccines such as measles, mumps, rubella, oral polio, smallpox, varicella and yellow fever are a weakened form of the "wild" viruses. These attenuated virus vaccines rely on limited replication of the virus in the host following vaccination. Immune responses induced are similar to those from natural infections and often these vaccines are effective after a single dose. However, such vaccines may cause severe reactions in some patients, which are often the result of the limited replication of the attenuated virus following vaccination. In contrast to attenuated live virus vaccines, inactivated (or killed) vaccines can not replicate, as their genetic material or overall structure are purposefully destroyed. These vaccines are safer than live vaccines but generally not as effective, requiring 3-5

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Table 1. Viruses that are major health threats

Virus	Disease
HIV	AIDS
RSV	Respiratory Infection
Hepatitis B	Liver Cancer
Hepatitis C	Cirrhosis/Cancer
Epstein Barr Virus	Lymphomas, Nasopharyngeal carcinoma
HPV	Cervical Cancer
Measles	Pneumonia (infants)
Influenza	Pneumonia

Abbreviations: HIV human immunodeficiency virus, RSV rous sarcoma virus, HPV human pappilomavirus.

doses as antibody titer falls over time. They lack the self-boosting qualities of live attenuated vaccines but are safer in the sense that the inherent dangers associated with virus replication are avoided. These vaccines are made as whole cell vaccines (such as Influenza, polio, rabies and hepatitis A) or as fractional or subunit vaccines such as hepatitis B. Subunit vaccines are based on the delivery of only a limited number of viral proteins, often the major protein in the capsid or envelope that is sufficient to confer protective immunity. These vaccines are an incremental step safer than inactivated vaccines because subunit vaccines can be prepared independent to the culture of replicating virus. Indeed, any remaining possibility of incomplete inactivation or batch to batch variation in the safety of the vaccine is eliminated. However, subunit vaccines have traditionally suffered from one important drawback; often single proteins when expressed and purified in the absence of other viral components are less immunogenic than those that are incorporated into infectious virus. This is probably because a proportion of this protein is present in a misfolded conformation relative to the native protein. Thus, more doses with higher amounts of antigen are required to achieve the same level of protection.

A major advance in subunit immunogen production has been assembly of proteins as virus-like particles (VLPs) using protein expression technology in yeast, insect or mammalian cells. VLPs are a highly effective type of subunit vaccines that mimic the overall structure of virus particles without any requirement that they contain infectious genetic material. Indeed, many VLPs lack the DNA or RNA genome of the virus altogether, but have the authentic conformation of viral capsid proteins seen with attenuated virus vaccines, without any of the risks associated with virus replication or inactivation.

VLP preparations are all based on the observation that expression of the capsid proteins of many viruses leads to the spontaneous assembly of particles that are structurally similar to authentic virus.[1-4] In practical terms, the fact that VLPs mimic the structure of virus particles usually means that VLPs should elicit strong humoral response and that lower doses of antigen relative to subunit vaccines are sufficient to elicit similar protective response. In addition to their ability to stimulate B cell mediated immune responses, VLPs have also been demonstrated to be highly effective at stimulating CD4 proliferative and = (CTL) responses.[5-7] This feature of VLP vaccines is likely to be a major contribution to their effectiveness in the field. It is also becoming increasingly clear that precise prime-boost strategies can be important to how effective vaccination is as a strategy to control disease. Therefore, the addition of VLP to the 'arsenal' of vaccine strategies for any disease extends the type of prime-boost regime that can be employed.

To date, VLPs have been produced for many different viruses that infect humans and other animals (see Table 2 and review).[8] One of the most striking features of this group is that it is extremely diverse in terms of the structure of the individual viruses. It includes viruses that have a single capsid protein, multiple capsid proteins and those with and without lipid envelopes. Clearly

Table 2 Baculovirus derived VLPs that have been tested as vaccines

VLP	Family	Proteins Expressed	Vaccine Tested In	VLP Refs.
Papillomavirus,	*Papillomaviridae*	1	Humans (licensed)	10,11,15-20,73
Norwalk and Norwalk-like viruses, Feline calicivirus	Calciviridae	1	Mice, cats, humans (Phase I)	26,27,74-78
Hepatitis E virus	*Hepeviridae*	1	Mice, cynomologous monkeys	28-30
Porcine parvovirus, mink enteritis parvovirus, Canine parvovirus, B19, adeno-associated virus	*Parvoviridae*	1	Pigs, dogs, mink	21-23,79,80,81
Chicken anemia virus, Porcine circovirus	*Circoviridae*	1, 2 (chicken anaemia virus)	Chickens	82-85
SV40, JC virus, murine polyomavirus	*Polyomaviridae*	1	Mice, rabbits (in vitro)	32,86,87
Polio virus	*Picornaviridae*	1 (polyprotein)	-	88
Bluetongue virus, Rotavirus	*Reoviridae*	4 (bluetongue) 2-3 (rota)	Sheep (bluetongue) Mice, pigs (rota)	4,35,42-49,89
Hepatitis C Virus	*Flaviviridae*	3	Mice, baboons	7,51,53
HIV, SIV, FIV, Visna virus, FeLV, BLV, Rous Sarcoma virus	*Retroviridae*	2	Mice, guinea pigs	2,3,50,90-96
Newcastle Disease Virus	*Paramyxoviridae*	1	Chickens	97
SARS Coronavirus	*Coronaviridae*	3	Mice (in vitro)	54
Hantaan virus	*Bunyaviridae*	3	Mice	98
Influenza A virus	*Orthomyxoviridae*	2-4	Mice	52,60,61
Infectious Bursal Disease virus	*Birnaviridae*	1	Chickens	34,52,99,100

Abbreviations: BTV Bluetongue virus, HIV Human immunodeficiency virus, SIV simian immunodeficiency virus, FIV feline immunodeficiency virus, FeLV feline leukemia virus, SV40 simian virus 40, rota rotavirus.

not all of the VLPs that are generated to date are appropriate vaccine targets, some VLPs have been generated to facilitate in fundamental understanding of virus assembly process, morphogenesis or architecture of viruses. However, an important point remains that the structure of the target virion is not limiting to the success of VLP production. Although various expression systems have been employed for VLP production, this chapter will mainly focus on insect cell culture produced VLPs that are being developed as candidate vaccines. The rationale behind this is that among all expression systems, insect cells, together with baculovirus expressing system, appear to be one of the most promising for VLP technology for development of viral vaccines (Fig. 1).

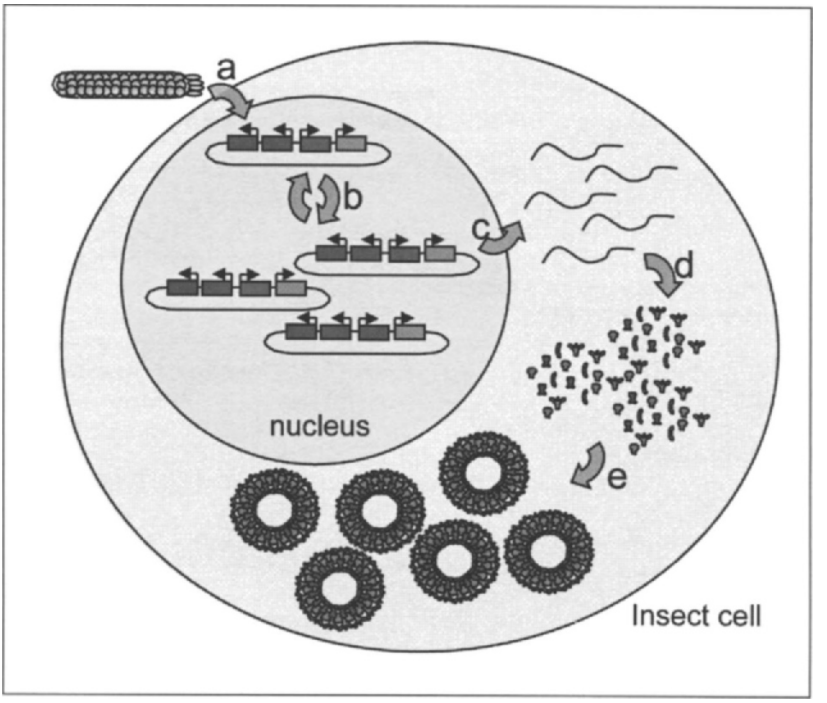


Figure 1. Key stages of intracellular assembly of VLPs using the baculovirus system. a) Baculovirus acts as a vehicle to efficiently deliver DNA, encoding recombinant proteins, to the nucleus of insect cells. b) Viral DNA is uncoated and replicates in the nucleus. c) Recombinant protein expression is driven by strong very-late viral promoters. d) Viral mRNA is used for the synthesis of recombinant proteins. e) VLPs are assembled by the interaction of proteins within the cytoplasm.

Insect Cells and Baculovirus Expression System as Preferred System for VLP Production

As stated above, a variety of protein expression systems are available to express recombinant proteins and particles. However certain criteria for generation of VLPs as prophylactic vaccines, particularly for human viral infection, must be considered. In order for a VLP to be a realistic vaccine candidate, it needs to be produced in a safe expression system that is easy to scale up to large-scale production. Table 2 shows baculovirus expressed/insect cell produced VLPs that have been demonstrated to be highly immunogenic and potential vaccine candidates. This insect cell-based protein production system has many advantages for VLP production. Firstly, extremely large amounts of correctly folded recombinant proteins can be produced in high-density cell-culture conditions in eukaryotic cells. Secondly, baculovirus expression systems have been developed for expression of multiple foreign proteins simultaneously from a single recombinant virus facilitating capsid assembly in each infected cell. Thirdly, as the insect cells that are used for vaccine production can be cultured without the need for mammalian cell derived supplements, the risks of coculture of opportunistic pathogens is minimized. Fourthly, the baculovirus used for recombinant protein expression has a narrow host range that includes only a few species of Lepidoptera and therefore represents no threat to vaccinated individuals. Finally the baculovirus system is amenable to scale-up for large scale vaccine production.[9]

VLPs Produced for Structurally Simple Non-Enveloped Viruses

For a number of nonenveloped viruses viral capsids are formed by only one or two major proteins and thus are relatively easy to manipulate for generation of VLPs by heterologous expression systems. Examples of these are the VLPs formed by the expression of the major capsid protein of Papillomaviruses, Parvoviruses, Calciviruses, Circovirses, Polyomaviruses and Hepatitis E virus (Table 2). All of these viruses are nonenveloped and have a single, virally encoded protein that forms the major structural component of the virion. Papilloma virus VLPs are among the most completely studied of this collection of VLPs and are at the most advanced stage with respect to production of a useful vaccine. VLP of Papilloma viruses are formed from the over expression of the major capsid protein L1.[10-12] These particles are highly immunogenic and are able to stimulate both humoral and cell mediated immune responses.[13-15] Human Papillomavirus (HPV) is the leading cause of cervical cancer. Globally, approximately 70% of all cervical cancer cases are associated with two serotypes of HPV, HPV-16 and HPV-18. VLPs produced in insect cells have been used successfully for Phase I and II human clinical trials in large numbers and were shown to be highly efficacious.[15-19] Moreover, GlaxoSmithKline's cervical cancer vaccine candidate (*Cervarix*™) targeting HPV 16/18 is currently undergoing Phase III clinical trials involving more than 30,000 women worldwide. In this Phase III randomized, double-blinded trial conducted in multiple centres in Denmark, Estonia, Finland, Greece, the Netherlands and the Russian Federation. All vaccinees received the HPV VLPs (HPV-16/18 AS04) as follows: 158 10-14 years old healthy girls and 458 15-25 years old young women received the candidate VLP vaccine according to a 0, 1, 6 month schedule and anti-HPV antibody titers were assessed. At month seven 100 per cent seropositivity was achieved in both groups for HPV 16 and 18 although average antibody titers for both HPV types were at least two-fold higher in 10-14 year-old girls. The vaccine was tolerated by all patients and no vaccine related serious adverse effects were detected. Further, the follow-up study clearly demonstrated the sustained efficacy of HPV-16/18 VLPs up to 4.5 years.[19,20] In conclusion, the bivalent HPV vaccine is highly immunogenic and safe and induces a high degree of protection against HPV-16 and HPV-18 infection and associated cervical lesions.

These studies are not only an important demonstration of the effectiveness of HPV VLP vaccine, and that multi-serotype VLPs are effective, but also highlight the fact that insect cell produced VLPs are a realistic alternative as human vaccines against viral disease. It should also be mentioned at this point that a tetravalent (HPV-6/11/16/18) VLP vaccine, Guardasil™ (Merk), produced in yeast cells was approved by FDA in June 2006 for use in women aged 9-26.

VLP vaccines for various diseases caused by parvovirus infections are also at an advanced stage although as yet none have undergone such large scale trials as those reported for HPV. Synthesis of major structural proteins VP2 of canine parvovirus (CPV) and porcine parvovirus (PPV) led to assembly of VLPs in insect cells.[21,22] Vaccination trials of CPV VLPs in dogs and PPV VLPS in pigs were highly encouraging.[21,23] In one efficacy assay dogs that received as little as or 10 μg or 25 μg of CPV VLP were completely protected from virus infection when challenged with virulent virus. Furthermore a single subcutaneous dose of 3 μg same CPV VLP with 50 μg ISCOM adjuvant was able to protect mink against challenge with the anti-genically similar virus, mink enteritis virus (MEV).[21] Similarly it has been reported recently that a single immunization with 0.7 μg of PPV (porcine starin) VLPs yielded complete protection in targeted animals against infectious PPV strains.[23] Indeed microgram doses of VLPs in gilts were not only highly immunogenic, but were also very efficient in preventing trans-plancental virus transmission and significantly reduced the number of reproductive failures. In addition, the feasibility of safe large-scale production of the porcine parvovirus VLP vaccine has been established complying with the European Pharmacopoeia requirements.[9]

Calicivirus studies have relied heavily on the production of proteins in heterologous systems mainly due to the fact that it is not yet possible to grow the virus in cell culture. Thus, VLP to Norwalk-like viruses have been extremely useful as sources of diagnostic antigen to monitor disease outbreaks. Norwalk virus VLP have also been shown to be effective at stimulating IgG, IgA and humoral responses in mice.[24,25] Preliminary Phase I trials in humans to test the safety and

immunogenicity of insect cell expressed Norwalk virus VLPs has confirmed that they are both safe and effectively stimulate IgG and IgA responses.[26,27]

VLPs for Hepatitis E have been assembled using a truncated form of the virus capsid protein.[28] In immunization studies in mice these VLPs were able to induce systemic and mucosal immune responses following oral administration.[28,29] Furthermore, oral administration of the Hepatitis E VLPs to cynomologous monkeys induced IgM, IgA and IgG responses and was sufficient to protect against infection and disease on challenge with virus.[30] Thus there is clear potential for the application of these VLPs as a vaccine for hepatitis E.

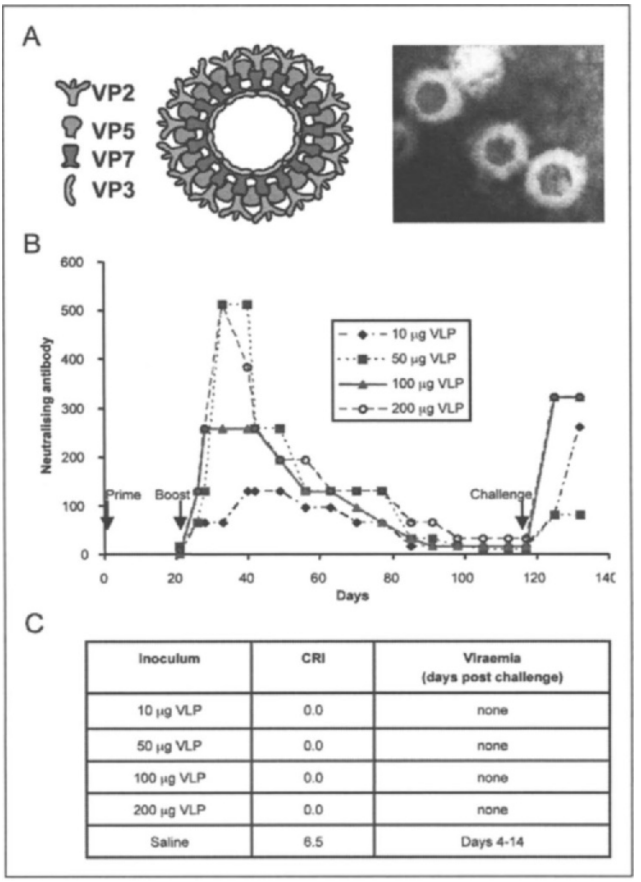
VLP preparations to Circoviruses and Polyoma virus are at a less advanced stage. VLP formation has been reported for Circovirus but as yet no serious attempt has been made at vaccine production. Vaccination of rabbits with VLPs for human JC virus in the presence of adjuvant allowed production of a hyperimmune serum that effectively neutralized infectious virus preparations.[31] However, in the absence of adjuvant there was no response. This pattern of response is unusual for VLPs in general, which often stimulate strong immune responses even in the absence of adjuvant. Indeed, VLPs of murine polyoma virus were able to stimulate a strong immune response in the absence of adjuvant when administered as a single 610 ng dose.[32] Intriguingly, these particles appear to be particularly stable with no alteration of particle morphology or reduction in immunogenicity even after 9 weeks storage at room temperature.[32]

VLPs of Structurally Complex Viral Capsids with Multiple Protein Layers

Viral particles that contain multiple interacting capsid proteins present more of a technical challenge than those that are formed by one or two major capsid proteins. Particularly, it is far more difficult if the assembling proteins of capsids are encoded by multiple discrete mRNAs, but not processed from a single polyprotein as in the case of picornaviruses. This is due the fact that for efficient assembly of a VLP the interacting capsid proteins must be expressed in the vicinity to each other, in other words in the same cell. Assembly of VLPs by processing of polyproteins have been achieved both for poliovirus[33] and for Infectious Bursal disease virus[34] using the baculovirus expression system. More complex assembly of multilayered, multiprotein VLPs have also been efficiently produced for the members of the *Reoviridae.* These viruses have capsids made up of concentric layers of different capsid proteins. Co-expression in insect cells of 2-4 of these capsid proteins, depending on the virus and the particle made, has allowed the production of VLP that are empty of the segmented dsRNA viral genome, but are otherwise indistinguishable from authentic viral particles.[4,35] The first member of the *Reoviridae* for which VLPs were described is Bluetongue virus (BTV), an insect transmitted animal virus. This remains the system in this family for which the largest variety of different VLPs and recombinant single antigen subunit immunogens made by baculovirus expression systems has been tested. In addition, the requirement for efficient co-expression of viral capsid protein in the same insect cell in this system has resulted in the development of baculovirus multigene expression vectors.[36,37] We will focus on this system in some detail as it highlights both the effectiveness of VLP vaccines and some of the technological advances that have been made for the production of VLP with complex architecture.

Bluetongue disease affects mainly sheep and cattle and is classified as an emerging disease in Europe.[38] The disease is caused by bluetongue virus, BTV, which has a multi-layered icosahedral structure formed by nonequimolar amounts of seven viral proteins (VP1-VP7). Three of these structural proteins (VP1, VP4, VP6) are dispensable for the formation of VLPs as they play only an enzymatic role in the virus transcription machinery.[38] The remaining four structural proteins (VP2, VP5, VP3 and VP7) are organised in two capsids. The inner capsid acts as a scaffold for the assembly of outer capsid that is responsible for cell entry and hence contains the major candidate for virus neutralisation.[38]

Expression of all four major structural proteins of BTV was achieved by construcing a baculovirus that simultaneously expressed all four proteins.[37] The advantage of this approach over co-infection with several baculoviruses each expressing a single protein is that equivalent conditions are achieved in all infected cells. Thus assembly of VLP is more efficient as expression



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Inoculum	CRI	Viraemia (days post challenge)
10 µg VLP	0.0	none
50 µg VLP	0.0	none
100 µg VLP	0.0	none
200 µg VLP	0.0	none
Saline	6.5	Days 4-14

Figure 2. Summary of production and testing of VLPs for Bluetongue virus. A) Left, cartoon showing the multi-layered structure of BTV VLPs. Right, electron micrograph of negatively stained BTV VLPs. B) Summary of neutralizing antibody response to VLP vaccination in Merino sheep. Sheep were vaccinated with two doses of VLPs with dose ranging from 10 µg to 200 µg as indicated. Neuralising antibody titre was followed for 117 days, at which point the sheep were challenged with virulent BTV. C) Table showing clinical reaction index (CRI) and length of Viraemia in sheep vaccinated with various doses of VLP and control. No signs of bluetongue disease or viraemia were detected in any of the VLP vaccinated animals.

is controlled at the level of the cell, rather than the level of the culture as is the case with mixed infections. BTV VLPs (Fig. 2) are structurally indistinguishable from virus particles but lack the segmented, double-stranded (ds) RNA virus genome normally present in infectious virus.[38]

Antibodies raised to purified BTV VLPs gave high levels of neutralizing antibodies against the homologous BTV serotype.[4] In subsequent clinical trials 1 year-old Merino sheep were vaccinated with various amounts (10-200 µg) of VLPs for BTV serotype 10. All vaccinated animals developed demonstrable neutralizing antibodies[39,40] and when challenged with virulent virus after four months of vaccination were completely protected from disease. In contrast, unvaccinated control animals developed typical BT clinical symptoms. Even at doses as low as 10 µg VLP was sufficient to protect animals from any signs of disease. Further efficacy tests were performed where VLPs from two different serotypes were combined to vaccinate the same animal. In these animals VLPs vaccination provided complete protection against the two vaccine serotypes and also partial

protection from challenge with related nonvaccine serotypes. The protective efficacy of vaccination in these trials extended over a long (14 month) period.[40] This observation raises the possibility that a broad spectrum vaccine against all 24 BTV serotypes is a possibility by combining VLPs from a relatively small number of serotypes.

The BTV system also demonstrates the efficiency of VLP vaccines relative to immunization with subunit vaccines based on dissociated antigens or unassembled recombinant antigens. In addition the assembled VLPs the two components of the BTV outer capsid, VP2 and VP5, were also prepared and tested in vaccination studies. While 100 μg VP2, the major serotype determining antigen, was only partially protective for a short duration (75 days) against virulent virus challenge, 50 μg of VP2 combined with 25 μg VP5 was protective.[41] In contrast, 10 μg VLPs (containing only 1-2 μg VP2) afforded a better level of protection for a much longer duration.[41] These studies demonstrate that assembly of antigens into VLPs results in a more effective immunogen than delivery of separately isolated proteins.

In addition to BTV, VLP have also been produced for rotavirus, another member of the *Reoviridae*. Intriguingly, VLPs formed from the two inner structural proteins alone of the rotavirus capsid have been shown to be effective immunogens in animal models.[42-48] Indeed in mice even intrarectal immunisation which induces a local mucosal response is sufficient for protection from rotavirus infection.[49] The data from these immunogenicity experiments are encouraging and it is possible rotavirus VLP may provide a viable alternative to the live virus vaccine for rotavirus.

VLPs from Viruses with Lipid Envelopes

Many pathogenic viruses such as Influenza, HIV and Hepatitis C are surrounded by an envelope, a membrane that consists of a lipid bilayer derived from the host cell, inserted with virus glycoprotein spikes. These proteins are the targets of neutralizing antibodies and are essential components of vaccine. Due to the inherent properties of lipid envelope, assembly of VLPs in insect cells for these viruses is a different type of technical challenge to those produced for viruses with multiple capsids. Nevertheless, efficient formation of VLPs of a number of enveloped viruses in insect cells has been reported. For example, VLPs of Hepatitis C virus, several retroviruses, SARS Coronavirus and influenza A have demonstrated correct assembly of the the lipid envelope with the glycoproteins inserted.[50-55] Indeed, for retroviruses, it has been possible to produce hybrid VLPs that contain the gag capsid protein from one virus (SIV) and the envelope protein from another (HIV)[56] in insect cells. Although none of the retrovirus derived VLPs are yet at the stage that they are being used in clinical vaccine trials, initial experiments in animal models are promising.[57,58]

VLPs for SARS Coronavirus as a basis for vaccination were produced rapidly following the SARS outbreak in 2002-2003.[54,55] However the control of SARS Coronavirus by epidemiological measures, continued lack of re-emergence of the virus, and difficulties working directly with the virus have severely limited the development of SARS VLPs as vaccine. Despite this, anti-serum raised in mice against insect cell derived SARS VLPs were able to neutralize a retrovirus pseudotyped with the SARS S protein (Fig. 3).

The Hepatitis C VLPs (Fig. 1) have been tested in mice and baboons and shown to be effective at stimulating both cellular and humoral immune responses.[7,53,59] In one experiment, 6-8 week old female BALB/c mice were immunized intramuscularly three times, at three week intervals with 20 μg insect cell derived HCV VLP, produced by co-expressing HCV coreE1-E2. Because of the lack of a suitable animal model for HCV infections a recombinant vaccinia virus expressing HCV structural proteins (vvHCV.S) was used as a model system. Vaccinated mice were challenged three days after the final immunization with vvHCV.S and then five days later the ovaries of infected mice were harvested and the vaccinia virus titre determined. Five out of seven vaccinated animals had no detectable vaccinia virus in the ovaries at this point. The remaining two animals had five logs lower vaccinia titres compared to control mice.[7] In addition, this study was able to demonstrate that the VLPs efficacy was based largely on its stimulation of $CD4^+$ and $CD8^+$ T-cell responses. A further study in baboons has demonstrated that the VLPs are well tolerated and can stimulate broad and long-lasting HCV targeted immune responses.[53]

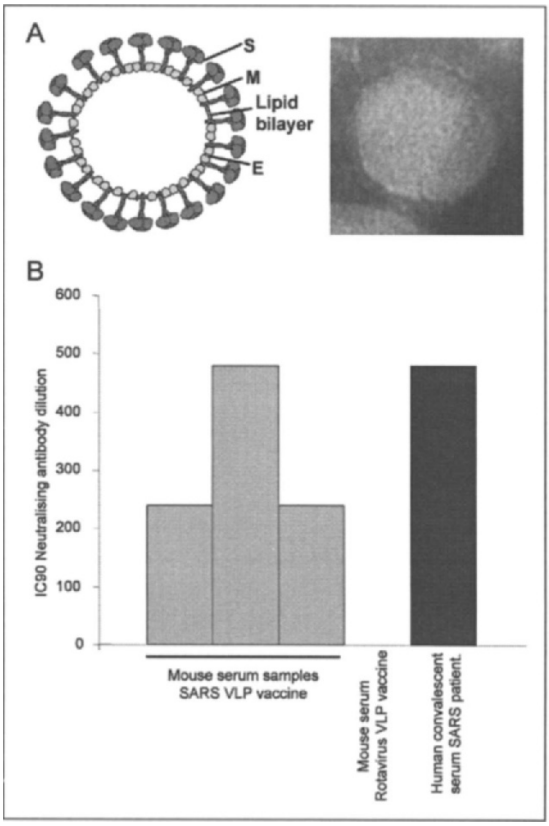

Figure 3. Summary of production and testing of VLPs to SARS coronavirus. A) Left, cartoon and right, electron micrograph of VLPs produced by co-expression of E, M and S proteins of SARS coronavirus. These VLPs were used to raise anti-sera in mice and the ability of these anti-sera to protect against infection with a SARS S protein pseudotyped lentivirus were assessed. B) IC90 neutralising antibody dilution for SARS S pseudotyped lentivirus, using sera from 3 mice immunized with SARS VLP, rotavirus VLP and serum obtained from a SARS convalescent patient.

To date, the most structurally complicated enveloped virus particle that has been used to generate VLP is influenza. VLPs for Influenza A H9N2 and H3N2 have been produced by other groups.[52] These studies have shown that expression of the major structural protein M1 alone is sufficient result in the budding of virus-like vesicles from insect cells.[52] Also, co-expression of M1 with M2, HA and NA leads to the assembly of influenza VLP and M1-HA and M1-HA-NA VLPs confer protection from lethal challenge with the same type influenza A in mice.[60,61] VLP production was also successfully achieved by co-expressing HA, NA, M1 and M2 from influenza virus A/Udorn/72 (H3N2) using a single recombinant baculovirus.[52] To date none of these influenza VLP have been tested in humans. However the potential that HA and NA could be incorporated directly into these VLP from circulating influenza strains without passage in tissue culture has particular advantage for the control of rapidly changing influenza A virus.

Future and Alternative Directions

In addition to the use of VLPs as direct immunogens, the efficiency with which they stimulate cellular and humoral responses has made them prime candidates as carrier molecules for the

delivery of epitopes, DNA and small molecules targeting other diseases. This has been facilitated by the excellent structural information that is often available for virus particles allowing rational design of vaccines where epitopes are exposed on the surface of the VLP. Many of the VLPs that have been developed as vaccines in their own right have also been tested as delivery systems for other molecules. It is not possible here to provide a full account of this approach, as the literature on delivery and display using VLPs is at least as large as that on VLP production for direct immunization (for review see ref. 62). However it is necessary at least to introduce this important area of VLP-based vaccine development. The use of VLPs as carrier molecules for epitopes for other diseases is not limited to those VLPs that are formed from the capsids of economically significant viruses. The reason that many VLPs make excellent carrier molecules for the delivery of epitopes in vaccines is most likely because the particulate VLP structure is readily taken up into antigen presenting cells and thus is able to prime long lasting CTL responses in addition to antibody responses.[6,63,64] Certainly accumulated evidence on VLP vaccines suggests that they are efficient at stimulating both cellular and humoral immune responses.[5-7,64-66] Notable work has been done in this area with both the hepatitis B core particles, human papillomavirus VLPs and parvovirus VLPs displaying T-cell specific epitopes from another protein on their capsid.[5,64-66,67] These studies demonstrate that like bacterial epitope display systems VLPs are efficient stimulators of MHC class I and class II responses.[63] Thus VLPs have great potential as epitope display systems for other diseases. The only major drawback for this approach is that the requirement of the capsid protein to assemble often constrains the size of the foreign sequence that can be tethered to the VLP. One approach that may be of use to overcome this constraint would be to link foreign protein sequences to capsid proteins in such a way that they extend the N or C termini of the protein and extend either inside or outside to particle.[68] Of course, this is only suitable where one or both termini of the protein are exposed on the inside or outside face of the capsid. So far, there are no VLP that we are aware of that have fully exploited the potential of this approach but it has been successfully employed for other protein-based particulate structures that are similar to VLPs in their stimulation of B-cell and T-cell responses and requirement for complex protein-protein interactions for particle assembly.[69-71]

Perspectives: Myths and Facts

Despite the accumulated evidence of the potential of VLPs as potent immunogens for many viral systems that we have discussed, there remains some resistance to the VLP approach as a general vaccination strategy for diseases caused by viruses. In part this is due to some high profile disappointing results for VLP vaccines in the early stages of development, for example an ineffective early vaccine for HIV based on Ty VLPs.[72] This example raises a point of caution for VLP vaccine designers. In general, VLPs stimulate efficient cellular and humoral immune responses but, as with any vaccine, they rely on the long term host response to be effective. VLPs designed to work in immunocompromised individuals need to overcome the same challenges to efficient immune response as any other vaccine approach. The notion that VLPs are ineffective vaccines is clearly a myth that is exploded by the imminent release of two new VLP-based HPV vaccines. Indeed, the accumulated data from the field suggests that VLPs are more effective than many other types of subunit vaccines, because they are more conformationally authentic and are safer than many live virus preparations because they are usually free of viral genetic material. VLP production does not appear to be limited to any one type of virus or virus family, nor is it significantly limited by the complexity of the virus particle.[8]

The use of insect cells as a protein expression system offers exciting opportunities for the synthesis of conformationally authentic VLPs that are formed from the intracellular assembly of multiple proteins expressed in the same cell. The advantage of this system over others used for protein expression is its capacity for industrial scale synthesis of large and multiple proteins and the fact that insect cells are the natural replication reservoir for many pathogenic viruses. Thus the basic cellular machinery that normally processes the infectious form of the virus is present within the expression system and available to produce authentic VLPs.

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