Small is beautiful: virus-like particles as vaccines

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Virus-like particles (VLPs) are nanoscale structures that are mimics of virus particles themselves. They are stripped of any infection risk but can perform like viruses in some tasks although they are unable to spread. They are inherently safe and may offer additional advantages when compared to the viruses from which they derive. If they are produced by a recombinant route, as is very common, they can be modified by alteration of the sequences used to add functionality their parental versions did not possess, such as staying in a particular conformation, carrying different cargoes or targeting specific cells. It is probably true that VLPs are ascribed more promise than may ever be realized but their positive attributes are very real, they have already appeared as successful products and their potential is certainly far from exhausted.

The term "virus-like particle" (VLP) is, perhaps too liberally, applied to many small aggregates whether they derive from viruses or not. To a degree this is justified as, indeed, viruses are generally small (most are ~50–150 nm diameter) with regular dimensions, so anything that resembles such structures may have the term applied to it. In recent years the VLP field has overlapped the emerging field of nanotechnology, which is a term applied to anything dependent on structures of ~100 nm diameter or less. The latter includes a range of structures with various intended functions, among them stimulation of the immune system, drug delivery, the encapsidation of nucleic acids or use as building blocks for larger arrays.

Although VLPs today generally mean a recombinant product made following the expression of a discrete number of virus-encoded proteins, it is important to note that VLPs are natural components of many virus replication cycles. The default infection for most viruses is acute. That is to say, a virus enters a cell and programs it to produce more virus particles. Speed and abundance are of essence as the virus has only a limited time before the immune system engages and starts to shut the infection down. Many viruses complete their replication cycle within a few hours, typically producing hundreds or thousands of new particles. So much virus protein is produced, notably the virus structural proteins that assemble virus particles, in such a short period of time that errors are inevitable. Coupled with this, as virus genomes are genetically limited, most virus particle assembly is self-directed by the structural proteins concerned. Hence incomplete or empty virus particles can partly form or form without anything inside them (Figure 1).

In a natural infection, aberrant particles are wasteful but tolerated as long as sufficient authentic virus is produced for onward transmission. Such particles, variously referred to as empty particles or defective interfering (DI) particles, qualify as VLPs and they occur in many virus types. Hepatitis B virus, for example, one of the smallest viruses, made of only two proteins, core and surface, produces both empty cores and aggregates of the surface protein during productive infection. Picornaviruses, a huge family of small RNA viruses that assemble from three structural proteins, also produce non-infectious empty capsids during their replication cycle (Figure 1). What these natural examples demonstrate is that VLP formation is inherent and not dependent on any complex cellular pathway. It follows that if the same proteins are produced in abundance by genetic means then VLPs assembly will similarly occur. Moreover, unless modification of the structural protein sequence occurs at a point which abrogates the ability to self-assemble then the same proteins with extended or swapped sequences will continue to form VLPs. No longer just mimics of the viruses from whose sequence they derive, such structures can act as scaffolds for the presentation of other polypeptides. Viruses generally demonstrate tropism, the particular property of binding one or other cell type as a result of the specific interaction with a virus receptor. VLPs that assemble to include the basis of tropism, the receptor binding protein, retain this ability and will also target specific cell types. In the normal virus the payload for delivery is the virus genome but if VLPs are assembled in its absence then the corresponding space can be filled with a cargo which is then delivered intact to particular cells by virtue of the natural tropism associated with the virus from which the VLPs were derived (Figure 2). The ability to form VLPs and their manipulation therefore offers a technology to safely mimic viruses themselves, to render non-assembling proteins into an assembled structure and to

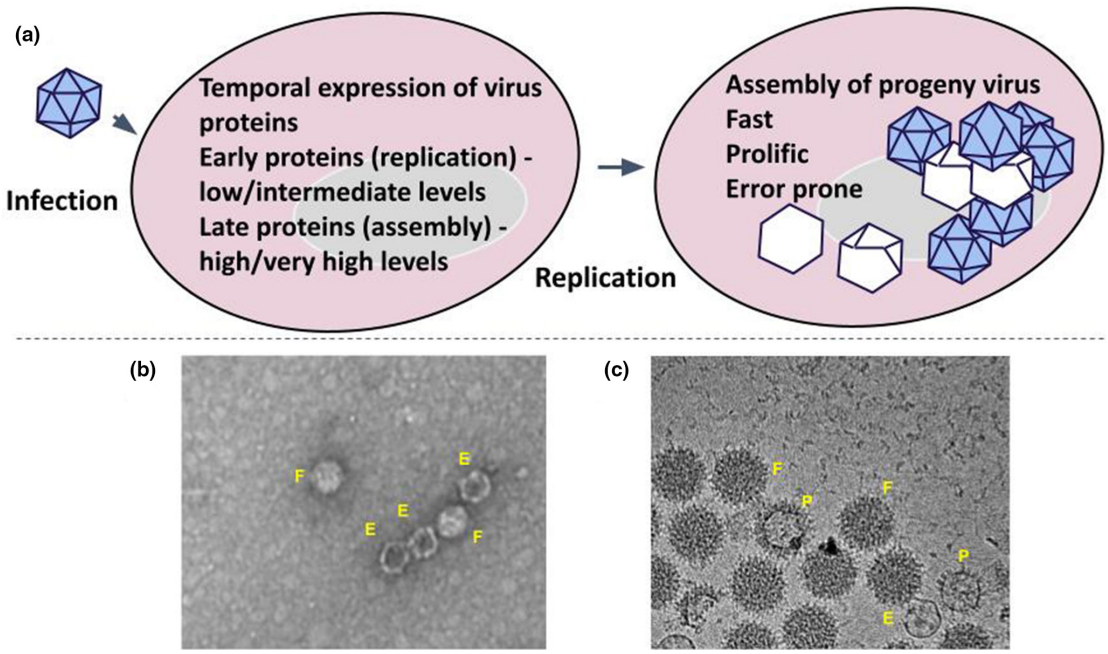

Figure 1. Virus-like particles are not new and occur as a side product in many virus replication cycles. (**a**) In the late stage of virus replication, the synthesis of the virus structural proteins is so prolific they make errors that result in empty particles. **(b** and **c)** Examples of empty particle assembly during the normal replication cycles of picornaviruses (**b**) and the more complex orbiviruses (**c**). F, full; E, empty; P, part assembled.

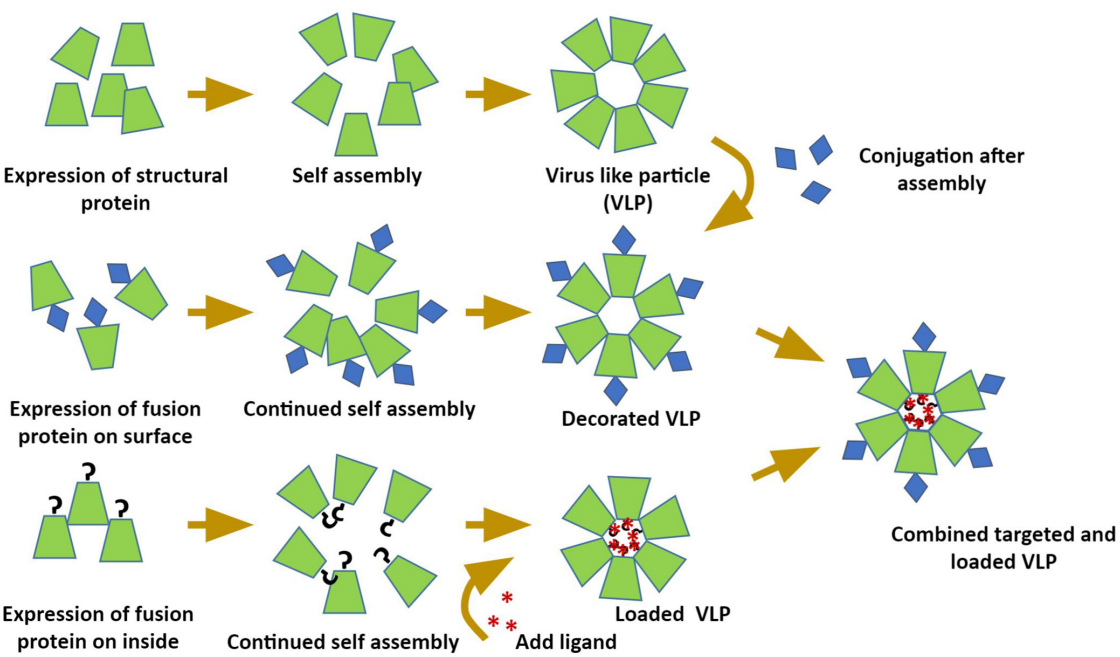

Figure 2. Types of virus-like particles. Top: all VLPs begin with the high-level synthesis of a protein, usually virus structural proteins, able to self-assemble. Assembly proceeds but in the absence of any other virus component, notably the genome, leading to the formation of a regular but empty VLP. Middle: if a sequence is inserted in the external face of the assembly protein and assembly is not compromised, a decorated VLP is the result. The same can be achieved by addition after VLP synthesis (top right). Bottom: a similar fusion but designed for the inside of the VLP can result in ligand capture and a loaded VLP. These possibilities can also be combined (bottom right).

offer protection and targeting to molecules that would otherwise be susceptible to degradation or dispersal. This utility is in marked contrast to the pathology that is the preoccupation of studies on the authentic virus.

Of all the potential uses for VLPs the most obvious and widespread is their use as vaccines. As noted, VLP assembly invariably involves the structural proteins of the virus particle, the same proteins that stimulate immunity during natural infection. In consequence, a VLP can act to stimulate the same immunity as that engendered by infection. The ability to produce VLPs by the expression of select viral proteins means there is no risk during manufacture, even for viruses that would otherwise require high containment. Manufacture may still be difficult or expensive but the VLP approach removes an infection risk that would be otherwise severely limiting. There are other reasons too for why VLPs are advantageous, as the structure of the VLP, with its repeating copies of one or a few virus proteins, stimulates immunity to a greater extent than the same material in disassembled form. An early step in the generation of immunity is the engagement of any pathogen by the B-cell, which carries on its surface a low affinity but very broadly reactive receptor (the BCR). The "match" between the foreign protein and the BCR triggers internal signalling that marks the beginning of the true immune response and this interaction is much more potent if multiple rather than single BCRs are engaged. A VLP naturally causes clustering of the BCR that single copies of the same proteins could not (Figure 3). A further step, internalization and degradation of foreign proteins for presentation to the awakening immune cells, is also enhanced as a VLP clearly delivers far more material than would a single copy of each of its constituent parts.

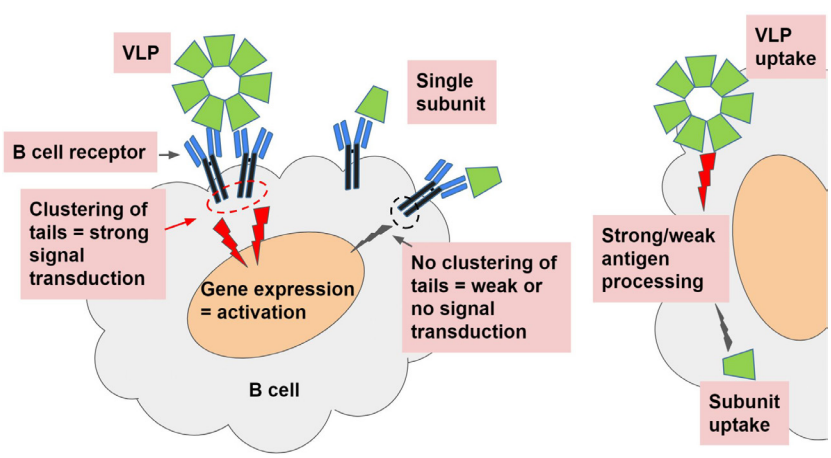

Figure 3. Pathways by which VLPs stimulate immunity. The multivalent nature of the VLP can cross-link the B-cell receptor on binding leading to clustering of the tails and strong signal transduction to activate the cell. Single subunit binding cannot do the same as cross-linking is not possible. On entry into antigen-presenting cells the mass of the VLP and its potential to be "seen" by innate sensors surpass that possible with the subunit alone.

These processes mean that VLPs represent not only a safe and manufacturable form of a vaccine but, on a weight for weight basis with individual virus proteins, a vaccine that is more able to generate the immunity associated with protection.

This concept is ably demonstrated by reference to some of the successful VLP vaccines now in use. The first of these (HBV), for hepatitis B, is a slight cheat. The basis of HBV protection is immunity to the surface antigen (S) and this protein was an early exemplar of the expression of viral proteins in recombinant expression systems. The universality of the genetic code means that the sequence of HBV S, when appended to the correct genetic control elements, produces the S protein in simple, easy to grow organisms like yeast, from which it can be purified by standard biochemistry. Fortuitously, purified S protein assembles into small aggregates that resemble VLPs even though the basis of the virus particle, the core protein, is not present. This assembled material is a very good vaccine and has been used worldwide since the mid-1980s. The HBV core meanwhile has been used widely as a scaffold for the development of many other vaccines, candidates for malaria and HIV among them. Initial work required the formation of a fusion protein in which the sequence of interest, the target for immunity, was genetically coupled to the HBV core protein resulting in a single polypeptide which assembles into a VLP. This is not always successful, however, as the sequence introduced sometimes folds on translation into a structure that prevents the HBV core from self-assembling. More recently, novel coupling technologies have been applied so that cargoes can be conjugated to the VLP after synthesis, which should result in a greater range of possibilities.

A second well-known example of a successful VLP-based vaccine has been the vaccine against human papillomavirus, the virus associated with cervical and other cancers. In this case, although the virus itself uses two proteins to form the particle, L1 and L2, L1 alone is sufficient to form a VLP. The ability to form a VLP revolutionized the vaccine possibilities for HPV as the virus itself cannot be grown in bulk, a case of the VLP providing not only a structural mimic but also a means to an end that had, hitherto, not been attainable. The expression technology in this case is either yeast or insect cells, both suitable for large-scale manufacture, and current vaccines are a mixture of up to nine serotypes providing protection against most circulating viruses of concern. Less well known but equally successful in the animal vaccine market is a vaccine for porcine circovirus type 2 (PCV2) which uses a VLP consisting of a single virus protein. Many other VLP-based vaccines are also in development.

Although VLPs have been successfully deployed as vaccines it would be wrong to conclude that they

are without issues. The immune stimulation that their polyvalent nature affords still does not mimic the immune triggering induced by the natural infection in some cases, and several boosts, or the inclusion of an adjuvant, may be needed to generate full protection. Similarly, manufacturing costs are not trivial as yields are variable and the purification extensive. VLPs can also exhibit instability with a tendency to disassemble on storage, meaning that cold chains may be required for their deployment. It is noteworthy in relation to the pandemic that VLP vaccines have not been among the front runners for a protective vaccine, despite the fact that coronavirus VLPs are readily formed from only two or three structural proteins. A balanced view would be that VLPs occupy a now well-established position within a range of vaccine technologies and their use will continue to grow. However, the choice of whether to make a VLP or not will depend on the particular vaccine required. ■

Further reading

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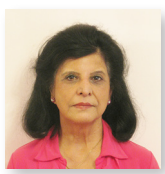

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