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Virus-like particles—universal molecular toolboxes

Christine Ludwig[1] and Ralf Wagner[1,2]

Virus-like particles (VLPs) are highly organised spheres that self-assemble from virus-derived structural antigens. These stable and versatile subviral particles possess excellent adjuvant properties capable of inducing innate and cognate immune responses. Commercialised VLP-based vaccines have been successful in protecting humans from hepatitis B virus (HBV) and human papillomavirus (HPV) infection and are currently explored for their potential to combat other infectious diseases and cancer. Much insight into VLP-mediated immune stimulation and optimised VLP design has been gained from human immunodeficiency virus (HIV)-derived VLPs presenting promising components of current AIDS vaccine approaches. Owing to their unique features, VLPs and virosomes, the *in vitro*-reconstituted VLP counterparts, have recently gained ground in the field of nanobiotechnology as organic templates for the development of new biomaterials.

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Introduction

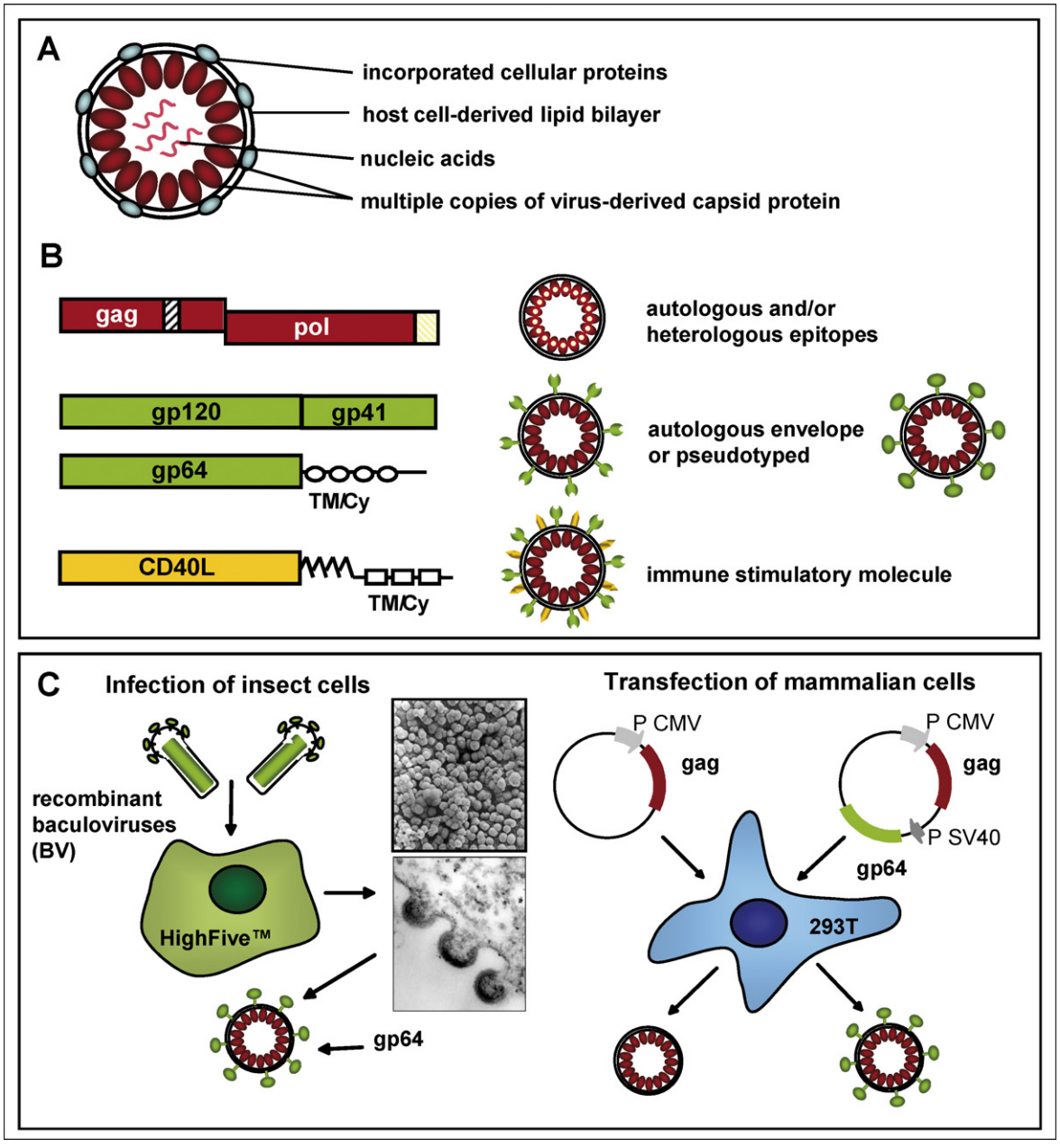
Viruses are vehicles of 15–400 nm in size engulfing their genetic information with a protein-based coat built of many spontaneously aggregating homogenous subunits and sometimes surrounded by a lipid bilayer derived from host cellular membranes. Viruses have evolved within a wide variety of eukaryotes (animals, plants, protists, fungi such as yeast) as well as prokaryotes (bacteria, archaea), those infecting the latter referred to as phages. According to the respective host spectrum, viruses have developed diverse shapes and replication strategies in order to survive and replicate within a hostile environment. However, not all vehicles assembled from virus components contain nucleic acids that are obligatory to establish productive infection. These virus-like particles (VLPs) representing empty shells are nevertheless still capable of entering target cells and are thought to be released from infected cells to entice host immune responses from the infectious viral offspring. This phenomenon has been exhaustively described for cells infected with hepatitis B virus (HBV) that mass produce empty 22-nm particles solely composed of the small HBV-derived surface antigen (HBsAg). Since these HBsAg particles have been isolated and characterised for the first time [1], fast-growing knowledge of VLP functions and structure combined with the accomplishments in genetic engineering have revealed manifold opportunities to exploit VLPs for various fields of molecular biology. Especially their natural immunogenic properties make them attractive candidates for vaccine strategies still ranking first among molecular VLP-based applications. During the past years VLPs have also established themselves in other branches of biotechnology taking advantage of their structural stability and tolerance towards manipulation to carry and display heterologous molecules or serve as building blocks for novel nanomaterials. These nearly unlimited possibilities have popularised VLPs within the scientific community as one of the most favourite tools of today's synthetic biology. This Review will therefore discuss the role of VLPs in current vaccine concepts in the light of their distinctive immunogenic potential and highlight recent achievements in the prospering field of virosome and nanobiotechnology.

VLPs as compounds of advanced vaccine strategies

Before 1969 all anti-viral vaccines were either based on inactivated viruses (e.g. rabies vaccine), attenuated strains of pathogenic wild-type viruses (yellow fever or polio) or were using closely related but non-pathogenic virus strains to induce a protective immune response against the pathogenic relative—like smallpox vaccine containing the innocuous vaccinia virus. Since HBsAg-based particles were first discovered in blood samples of hepatitis B patients and were soon developed into a potent vaccine against the cognate virus infection [2], subviral particles have rapidly found their way into modern concepts of vaccine design.

On the basis of their particulate nature (Figure 1A), VLPs provide an inherent advantage over soluble antigens that have been shown to fail in several vaccine approaches owing to weak immunogenicity or instability. Since VLPs are not infectious and do not replicate, they also represent a safer alternative to attenuated viruses, which have succeeded to protect from yellow fever, polio, measles, mumps and rubella, but have clearly failed in other

Figure 1



VLP-based strategies for vaccine design. **(A)** Schematic presentation of a virus-like particle composed of self-assembled virus capsid proteins that are engulfed by a host cell-derived lipid membrane with integrated cellular proteins; random packaging of cellular nucleic acids is indicated. **(B)** Vector constructs used to express chimeric HIV VLPs; top: VLPs assembled from Gag or Gag-Pol proteins present endogenous (hatched box) epitopes; additional foreign epitopes can either be inserted or fused (yellow box); middle: VLP-display of incorporated envelope proteins by co-expression of either autologous (gp120/gp41) or heterologous (e.g. gp64) envelopes, the latter resulting in pseudotyping; bottom: co-stimulatory molecules (CD40L) can be anchored on VLPs via a transmembrane domain (TM); Cy, cytoplasmic domain. **(C)** Production of VLPs in different expression systems. Left: infection of HighFive^{TM} insect cells with recombinant baculoviruses (BV) expressing respective capsid proteins; VLPs budding from insect cells (electron microscopy images) carry BV gp64; right: co-transfection of mammalian 293 T cells with a *gag* encoding plasmid; co-expression of gp64 results in pseudotyping of Gag VLPs. P CMV, cytomegalovirus promoter; P SV40, Simian virus 40 promoter.

infections as formerly shown for attenuated versions of human immunodeficiency virus (HIV), which reconverted into pathogenic species over time (discussed in references [3,4]).

VLP vaccine production

The demands for higher yields of the HBsAg-based subviral vaccine than those recovered from blood samples soon turned towards other more efficient production

systems exploiting the possibilities of recombinant DNA technology. By virtue of HBsAg to self-assemble into particulate structures, respective particles were efficiently released from recombinant yeast cells allowing high-yield production and safer purification of the vaccine [5]. Apart from yeast spheroblasts, other systems have been exploited for VLP expression. The most prevalent ones include the vaccinia virus expression system, transfection of RNA- and codon-optimised DNA into mammalian cells or infection of insect cells with recombinant baculovirus. VLPs designed for mucosal vaccination can also be efficiently produced in gut bacteria such as highly attenuated *Salmonella* or *Lactobacillus* strains [6,7]. Besides, green plants like tobacco, potato or tomato offer an efficient and inexpensive method for VLP production by infection with plant-specific viruses, such as, for example the tobacco mosaic virus [8,9•]. Of note, plant-produced VLPs have been shown to be highly immunogenic upon oral ingestion of VLP-containing plant material [10].

During the past decade, subviral particles of different origins have been considered as potential vaccines for cognate virus infections including human immunodeficiency virus, human papillomavirus (HPV), Norwalk virus (NV), rotavirus or parvovirus. A very comprehensive overview of these VLP-based vaccine approaches is given in references [11,12]. It is noteworthy that VLPs assembled from HPV major capsid protein L1 in yeast were capable of inducing protective immune responses against HPV subtypes 16 and 18 causing cervical cancer in humans, thus resulting in a safe, well tolerated and highly immunogenic vaccine that received approval for marketing in 2006 [13]. A competing product produced in the baculo-expression system has been approved recently and claims to be even more efficient concerning long-term immune responses triggered by the potent adjuvant AS04 [14]. Apart from HBV or HPV, the enduring efforts to develop a safe, prophylactic AIDS vaccine have called VLP-based strategies into action.

VLPs, successful perspective for an AIDS vaccine?

Early conventional attempts towards developing a protective HIV vaccine on the basis of soluble HIV-1-derived envelope protein gp120 have been rather disappointing. Since particulate antigens had been demonstrated to induce better cellular and humoral immune responses than soluble antigens, the detection that HIV-1 Pr55Gag polyprotein self-assembles into particulate spheres provided a new rationale for generating a Gag-based VLP vaccine [15,16]. Gag is today one of the two most common antigens expressed for vaccine approaches, and respective VLPs can be efficiently produced in mammalian or insect cells (Figure 1C). First immunisation analyses with Gag VLPs yielded strong Th1-biased humoral and cellular immune responses in the absence of adjuvants and induced Gag-specific cytotoxic T lymphocytes (CTL) in mice and macaques [17,18]. Today it is a widely held belief that both, a strong cell-mediated immune response and cross-reactive neutralising antibodies mainly directed towards conserved epitopes of the envelope (Env) protein are essential to achieve protection from HIV infection. Hence, current approaches are being extended to novel VLP designs addressing both aspects to induce an integrated immune response.

Tailor-made VLPs for improved immunogenicity

A good vaccine has to fulfil certain criteria concerning immunogenic potential, cell or host tropism, route of administration and uptake. In this regard, not all attempts to use VLPs built of structural proteins derived from the cognate virus have yet been successful in eliciting effective immune responses. To allow further reconfiguration of the native structures and adaptations in VLP design, the self-assembling antigens such as HIV Gag, HBsAg and HBcAg (hepatitis B core antigen), parvovirus VP2 capsid protein or p1 protein of yeast transposon Ty have been extensively studied in order to identify domains dispensable for particle formation to clear a space for insertion of more relevant sequences. In case of HIV-1 Gag, parts of the matrix and capsid proteins or the carboxyl-terminal p6 moiety can be deleted without affecting particle assembly and then be replaced by or fused with heterologous sequences either derived from other regions of the cognate virus or from a foreign virus, thereby allowing efficient display of corresponding epitopes in the Gag particle context. Since multi-epitope vaccines have been shown to be more successful in inducing broad immune responses, Gag-based VLPs were soon extended to contain larger regions of the virus, and thus present more epitopes to the immune system. In this respect, the discovery that HIV full-length Gag-Pol precursors comprising additional enzymatic activities were also capable of forming particles was very helpful to develop a second generation of multi-epitope VLPs (Figure 1B).

Compared with these type-1 VLPs, which are capable of eliciting strong CTL responses towards the inserted epitopes, type-2 VLPs exposing incorporated envelope proteins on their surface have been more successful in inducing humoral antibody responses (reviewed in detail in reference [19••]). Aiming towards induction of broadly cross-neutralising antibodies, numerous Env-specific modifications have been conducted to achieve either maximal incorporation of Env molecules (by truncation or fusion with heterologous transmembrane domains), to improve surface-presentation of native conformations of Env (e.g. by trimer-stabilisation) or distinct exposition of conserved gp41 epitopes [19••,20]. In this context, HIV-Env immunogenicity has also been extensively analysed in the monkey model using Simian immunodeficiency virus as a carrier resulting in chimeric SIV-HIV viruses (SHIV) [4].

Apart from Env-directed approaches, pseudotyping with fusogenic heterologous envelopes has proven to provide additional immunogenic benefit by augmenting VLP uptake via antigen presenting cells (APCs). For example, incorporation of surface glycoprotein from vesicular stomatitis virus (VSV-G) into HIV VLPs significantly increased Gag-directed antibody and T cell responses in mice and reduced viremia in challenged monkeys [21]. In other cases, where a less broad tissue tropism is needed, the baculovirus-derived envelope glycoprotein gp64 shown to mediate efficient transduction of mouse cells *in vivo* might provide a less toxic alternative to efficiently pseudotype VLPs [22]. Last but not least, VLPs pseudotyped with heterologous Envs can be simply used as platforms to efficiently present the incorporated Env proteins to the immune system. As an example, Gag VLPs pseudotyped with equine herpesvirus type 1 (EHV-1)-derived glycoprotein gp14 elicited protective immune responses against EHV-1 challenge in mice upon intranasal application [23] emphasising the immunogenic potential of particulate antigens.

To further enhance the immunogenicity of recombinant Gag VLPs, they have been equipped with additional co-stimulatory molecules such as influenza hemagglutinin (HA) or cholera toxin subunit B, both shown to significantly stimulate mucosal immune responses that have to be essentially induced to fend the virus at the gateway. Increased stimulation of mucosal cellular immune responses has also been achieved by expression of VLPs in gut bacteria such as *Salmonella* as vehicles for the *gag* gene [6]. Furthermore, an induction of Env-specific IgA and IgG responses following intranasal application of Gag-Env VLPs has lately been reported [24], underlining the impact of the route of administration. Quite recently, Skountzou *et al.* [25•] have demonstrated that the adjuvant properties of chimeric SIV VLPs could be significantly enhanced in immunised mice when loaded with co-stimulatory molecules GM-CSF (granulocyte-macrophage colony-stimulating factor) or CD40 ligand (CD40L). A schematic illustration of the established strategies to construct and equip chimeric VLPs is given in Figure 1B.

Eventually, expression of VLPs from DNA plasmids encoding RNA and codon-optimised *gag* genes or lentiviral vectors has become apparent as an efficient strategy to design highly immunogenic HIV vaccines (reviewed in reference [4]). VLP antigens expressed from DNA-transfected cells are capable of entering into both, the major histocompatibility complex (MHC) class-I and class-II processing pathways thereby stimulating $CD8^+$ and $CD4^+$ T cell as well as B cell responses. Compared with soluble antigens, DNA vaccines are easier to produce and deploy the positive effects of live attenuated vaccines to directly stimulate MHC class-I restricted CTL responses. Support for this hypothesis has been provided in a recent study published by Bellier *et al.* [26]. The authors demonstrated efficient expression of murine leukemia virus (MLV) Gag-Env VLPs from plasmid DNA *in vitro* and used these plasmo-retroVLPs to induce strong specific CTL responses towards displayed T cell epitopes, protecting mice from lethal virus challenge. Currently, VLPs are an inherent part of most multi-component status quo HIV vaccines, and one promising concept shown to induce broad long-lasting immune responses is a combination of DNA/VLP prime followed by booster immunisation with live attenuated vaccinia vectors [27]. We and others have previously summarised the recent achievements on the way towards a potent VLP-based HIV vaccine [3,4,19••].

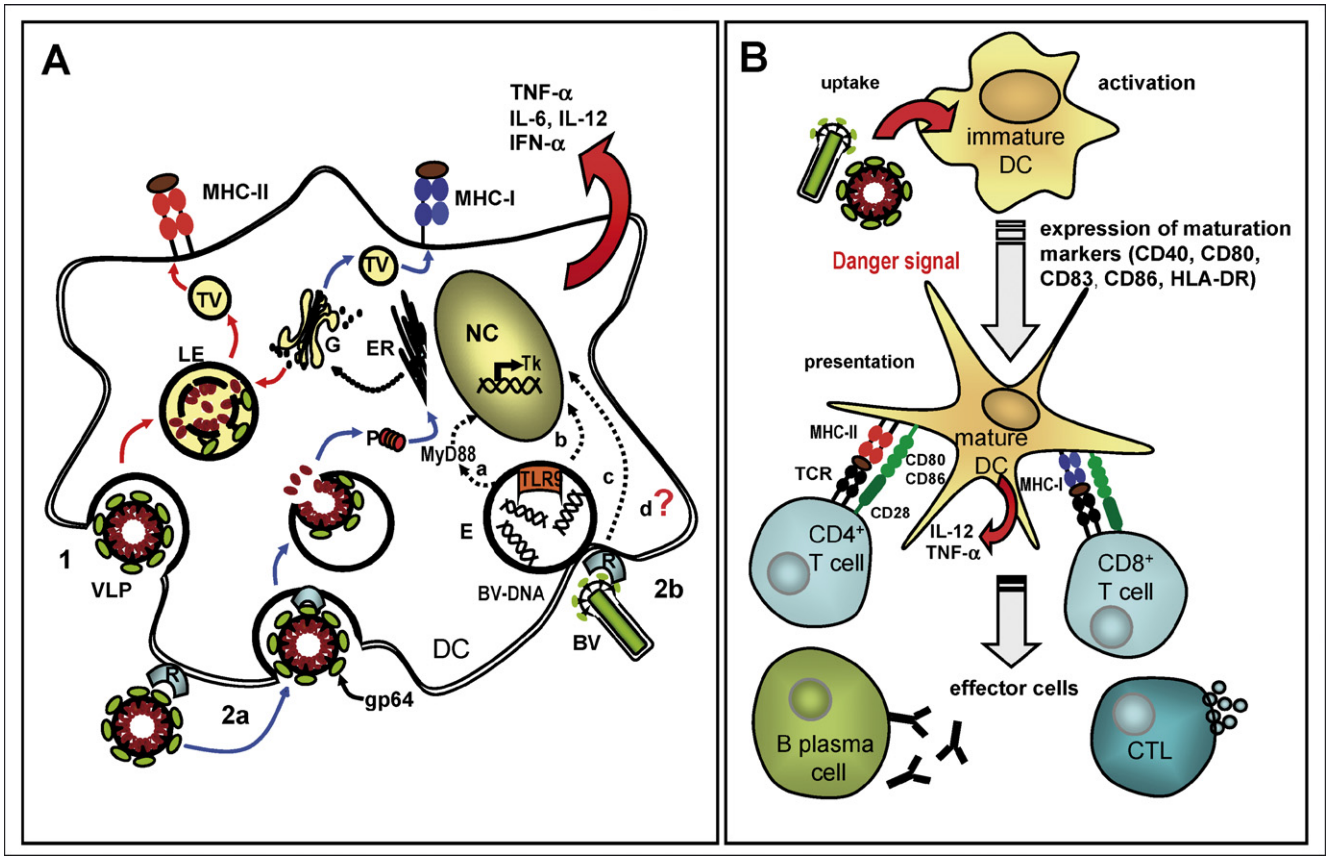
The enormous convertibility of VLPs illustrated by the numerous approaches to reconfigure particulate antigens for vaccines has also been successfully exploited in other areas such as gene therapy. A demonstration for this has been given in a recent study [28••], where VLPs carrying the HIV CD4 receptor complex and a cytotoxic compound are specifically targeted to HIV-infected Env-expressing cells via an inverse fusion process resulting in release of the compound and killing of infected cells. Such therapeutic VLPs might be useful tools in the future to attack long-time HIV reservoirs.

Immunogenic properties of VLPs

Compared with soluble antigens, which need to be co-administered with adjuvants in several booster injections to elicit protective immune responses, VLPs are capable of inducing strong cellular and humoral responses as direct immunogens (reviewed in reference [12]). VLP size appears to be favourable for uptake by dendritic cells (DC) via macropinocytosis and endocytosis that play a central role in activating innate and adaptive immune responses. A growing body of data indicates that Gag VLPs that *per se* contain many immunogenic epitopes are capable of stimulating cellular immune responses via both, the MHC class-I and MHC class-II pathway [19••] (see also Figure 2A). Owing to the repetitive particle structure, uptake of a single VLP feeds thousands of contained epitopes into the processing and presentation machinery of APCs, a process thought to be supported by the fusogenic activity and the lipid nature of VLPs. These assumptions are in line with recent findings that pseudotyping of VLPs with envelope proteins like VSV-G, thereby improving uptake via receptor-dependent fusion, increases epitope presentation via the exogenous MHC class-I pathway and subsequent CTL induction [21,29].

In principle, all established VLPs proved to be strong stimulators of the innate immune system. The extensive potential of VLPs to activate and mature DCs thereby triggering the expansion of numerous populations of immune cells *in vivo* has recently been diligently

Figure 2



Putative mechanisms of VLP-mediated stimulation of innate and cognate immune responses. **(A)** Model for the activation of dendritic cells (DCs) by baculo-derived VLP preparations. VLPs are taken up by DCs via endocytosis (**1**) directing antigen processing in late endosomes (LE) and presentation via the MHC class-II pathway (red path), or via receptor (R)-mediated fusion triggered by gp64 (**2a**) resulting in proteasomal antigen processing in the cytoplasm and subsequent presentation on MHC class-II (blue path). Baculoviruses (BV) are taken up by gp64-mediated fusion (**2b**). Danger signals from CpG-rich BV-DNA are recognised by endosomal (E) Toll like receptor 9 (TLR9) and transmitted via a MyD88-dependent (a) or independent (b) signalling pathway resulting in activation of transcription (Tk) and production of inflammatory cytokines and type 1 interferons. A TLR9-independent route of DNA recognition inducing production of IFN-α has also been described (c). Additionally, BV-derived components other than DNA might contribute to DC activation (d). G, Golgi; ER, endoplasmic reticulum; NC, nucleus; P, proteasome; TV, transport vesicle. **(B)** VLP-mediated maturation of DCs. Uptake of VLP/BV activates DC via danger signals resulting in upregulation of DC maturation markers. Mature DCs present VLP-derived antigens to naive $CD4^+$ and $CD8^+$ T cells via MHC class-I and class-II. Secretion of cytokines by DCs stimulates differentiation into B and T effector cells resulting in antibody release and cytotoxic T cell (CTL) responses.

investigated by Sailaja and *et al.* [30•]. However, VLPs used in this study were produced in the baculo-expression system. Actually, increasing evidence suggests that contaminating components in VLP preparations might play a crucial role in stimulating both, innate and cognate immune responses. The majority of VLP preparations currently applied are derived from infection of insect cells with recombinant baculoviruses (BV), which are popular owing to their power regarding expression of heterologous proteins, their incapability to replicate in mammalian cells, a low cytotoxicity and the absence of a pre-existing immunity in humans. Routine purification procedures like crude gradient centrifugation or ultrafiltration do not strictly discriminate between VLPs and BVs. As a consequence, VLP preparations are probably enriched by BVs potentially contributing to the adjuvant properties of VLPs (illustrated in Figure 1C). Indeed, wild-type BVs have been reported to induce strong innate immune responses upon intranasal inoculation capable of protecting mice from lethal challenge with influenza virus [31]. The associated secretion of inflammatory cytokines such as tumour necrosis factor (TNF)-α, interleukins (IL)-6 and-12, and type 1 interferons (IFN-α/-β) was shown to be at least partly induced via the Toll-like receptor 9 (TLR9)/MyD88 signalling pathway known to recognise internalised CpG-rich DNA from bacteria [32]. Indeed, it has been reported that BV DNA probably released into the cytoplasmic compartments containing TLR9 upon gp64-mediated membrane fusion has a CpG content similar to bacteria, which might be regarded as a danger

signal. However, residual IFN-α release upon BV stimulation in TLR9/MyD88 knockout mice indicated that other possible mechanisms might be involved in BV-mediated immunogenicity, as has been suggested for the surface glycoprotein gp64 owing to its distinct *N*-glycosylation pattern. A model for BV-mediated DC stimulation is provided in Figure 2A. The adjuvant properties of BV have been corroborated by recent findings showing that BV-induced humoral and CTL responses against co-administered antigens were triggered via IFN-α and β, which directly mature DCs thereby driving the differentiation of B and T cells into effector and memory cells [33••] (Figure 2B). In further support of this hypothesis, BV-produced HIV VLPs formerly demonstrated to elicit strong immune responses in Balb/c mice [34] were recently shown to mature monocyte-derived DCs associated with significant upregulation of surface maturation markers (CD40, CD80, CD83, CD86, HLA-DR) and increased release of Th1- and Th2-specific cytokines [35]. Apart from BV-derived VLP preparations, specific immunomodulatory effects have also been ascribed to yeast-derived particles, which have been shown to enter DCs via mannose recognition resulting in cross-priming of Gag-specific $CD8^+$ T cells [36]. In sum these data suggest that the respective system used for VLP production might significantly influence direction and outcome of the induced immune response. Albeit the described adjuvant properties of contaminating baculovirus- or yeast-derived components might substantially trigger the overall immunogenicity of VLP preparations, the development of such a vaccine raises fundamental safety concerns considering the associated regulatory complications. Basically, advanced procedures for production and notably purification of VLPs will be needed to obviate these unforeseeable side effects.

Lessons learned from combating the well-known viruses like HBV, HPV or HIV are constantly being carried over to newly emerging and yet less intensively studied virus diseases, for which VLP-based strategies might serve as attractive first trial tools to develop a protective vaccine. As an example, VLPs assembled from filovirus-derived matrix protein VP40 have been demonstrated to induce neutralizing antibody responses capable of protecting rodents from lethal challenge with Ebola or Marburg viruses, which cause severe hemorrhagic fever in humans [37,38]. Very recently, VLPs assembled from Severe Acute Respiratory Syndrome (SARS) coronavirus (SARS-coV)-derived S, M, and E proteins have been reported to elicit strong humoral and cellular immune responses in mice, thus providing a promising strategy to confine SARS in the near future [39]. On the basis of the accumulating knowledge of VLP function and the importance of diligent VLP design to perform in a vaccine setting, old VLP formulations, such as rabbit hemorrhagic disease virus (RHDV) particles, protecting rabbits from the cognate virus infection, are being discovered for new purposes. RHDV VLPs chemically modified to allow covalent conjugation of peptides and proteins have lately been shown to induce strong humoral and cellular immune responses towards the attached antigens [40]. This illustrates the potential virtue of combining biological and chemical techniques to overcome restrictions in size or accessibility of VLP-inserted epitopes.

VLPs versus virosomes

A second class of stable spheres resembling VLPs and with a wide current range of applications is referred to as virosomes, representing preformed virion-like liposome-based complexes with integrated surface glycoproteins for receptor-mediated endocytosis. Like VLPs, virosomes possess excellent adjuvant properties and have been established for many viruses like HIV, EBV, Sendai virus or rabies either for vaccination purposes or delivery of nucleic acids, drugs or heterologous antigens (reviewed in reference [41]).

The IRIV hype

Among all circulating virosome species, the immunopotentiating-reconstituted influenza virosomes (IRIVs), which are produced by detergent solubilisation of influenza viruses and then reconstituted with influenza envelope proteins HA and neuraminidase are certainly most prevalent [42]. HA stabilises the liposomal particles mainly composed of phospholipids and phosphatidylcholine and binds to sialic acid expressed on APCs, thus allowing receptor-mediated fusion followed by subsequent presentation of integrated antigens via MHC class-I and surface-exposed antigens via MHC class-II. IRIVs are part of licensed hepatitis A virus and influenza vaccines and have been shown to efficiently boost humoral and cellular immune responses towards all kinds of integrated antigens such as tumour markers, malaria-specific peptides, Leishmania-derived carbohydrates or toxoids [43–47]. Even cytotoxic drugs, like doxorubicin are efficiently delivered to tumour cells by virosomes displaying polyethylene glycol-conjugated receptor-specific antibodies that might pave the way for novel cancer therapy strategies [48].

Albeit virosomes have been demonstrated to induce longer lasting immune responses than conventional adjuvants while igniting fewer adverse side effects [49], their adjuvant properties can be further increased by integration of co-stimulatory molecules as has been demonstrated for an IRIV-based cancer vaccine co-assembled with CD40L [50]. Alternatively, strong CTL responses demonstrated to be crucial for tumour growth control could be induced by means of recombinant IRIVs specifically targeted to plasmacytoid DCs [51]. Apart from representing antigen delivery and display platforms, virosomes are excellent carriers of nucleic acids that form stable complexes with the liposome spheres. Cusi *et al.* [52] have formerly shown that virosome-mediated targeting of mumps virus DNA to

APCs induces specific CTL responses suggesting efficient expression and presentation of the encoded mumps antigens. To protect the active DNA component from cellular nucleases, De Jonge *et al.* have recently developed a method to encapsulate plasmid DNA into virosomes via a short-chain phospholipid [53•]. Another innovative approach has used virosomes to channel therapeutic siRNA complexed with cationic lipids into target cells [54].

Virosomes are becoming more and more popular in modern vaccine concepts, since they combine the safety and flexibility of subunit vaccines with the biological and immunogenic properties of VLPs. However, the barriers between VLPs and virosomes as by definition are beginning to melt. Recently, a novel species designated immunovirosomes has been developed where Moloney leukemia viruses were mixed with liposomes and conjugated with a receptor-directed antibody to allow tissue-specific targeting of a therapeutic antigen [55].

VLPs as scaffolds in nanoparticle biotechnology

Nanoparticles basically encompass all particulate structures ranging between 5 and 100 nm in size. Especially the capability of virus-derived nanostructures to assemble into highly organised regular arrays together with their susceptibility to accept a wide range of chemical modifications has offered new perspectives for the usage of VLPs in manifold biotechnological processes. Wang *et al.* have formerly exploited the possibility to chemically modify mutant cowpea mosaic virus (CPMV) particles – which are highly stable and offer multiple reactive sites – by exposing sulfhydryl groups in order to attach fluorescent dyes and gold clusters [56]. CPMV was also shown to efficiently self-assemble into monolayers at perfluorodecalin–water interfaces, which can be cross-linked to form a robust membrane [57]. Likewise, the tobacco mosaic virus (TMV)-derived capsid protein has proven to be a suitable scaffold for extensive chemical modification allowing assembly of nanobiopolymers (summarised in reference [9•]. Yet another study has described the usage of recombinant M13 phage particles as organic templates to polymerise nanowires as building blocks for semiconductors or magnetic materials [58]. Likewise, the need for new nanowire material for the construction of smaller lithium ion batteries has drawn the attention to filamentous M13 phages, which were modified to surface-expose tetraglutamate to nucleate metal ions such as the lithium active compound cobalt oxide [59]. In all these approaches, chemically modified VLPs maintained their structural integrity that is a prerequisite for VLPs to prevail in the future world of nanoscience.

Conclusions

On the basis of their flexibility and stability, simple production and distinctive immunogenic properties, VLPs offer vast opportunities of application in the fields of vaccine development, gene therapy as well as nanobiotechnology. Many lessons have been learned from VLP-based technologies, which will certainly find themselves confronted by new challenges in the near future, such as the demand for innovative biomaterials or potent vaccines for newly emerging diseases. In this light it appears almost ironical that viruses as such may serve a good purpose in the biotechnological era exploiting their weapons to beat them at their own game.

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Conflict of interest

The authors state that no conflicts of interest exist.

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- • of special interest
- •• of outstanding interest

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This comprehensive Review article plausibly illustrates the challenges VLP-based HIV vaccine approaches had to take over the past 10 years. On the basis of the history of HIV Pr55Gag as the elementary antigen for VLP production, the authors have compiled all relevant former and novel strategies for vaccine design with a special focus on the immunogenic properties of VLPs.

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In order to enhance the immunogenic properties of chimeric SIV Gag-Env VLPs, the authors have incorporated the immunostimulatory molecule CD40L. Alternatively, GM-CSF was anchored in the VLP membrane by means of glycosylphosphatidylinositol (GPI). Both particle species were demonstrated to strongly enhance CD4$^+$ and CD8$^+$ as well as humoral immune responses towards SIV Env.

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This is the report about an unconventional method that uses HIV VLPs to specifically target and eliminate HIV-infected cells. For this purpose, the authors have created a recombinant VLP displaying the cell-derived HIV-specific CD4 receptor complex and carrying thymidine kinase (TK) of herpes simplex virus-1, which has proven to be highly cytotoxic under ganciclovir treatment causing chain termination during replication. This TK is delivered by VLPs via an inverse fusion process to latently infected cells that express HIV proteins and surface-expose Env upon cytokine stimulation, resulting in efficient cell killing.

29. Marsac D, Loirat D, Petit C, Schwartz O, Michel ML: **Enhanced presentation of major histocompatibility complex class I-restricted human immunodeficiency virus type 1 (HIV-1) Gag-specific epitopes after DNA immunization with vectors coding for vesicular stomatitis virus glycoprotein-pseudotyped HIV-1 Gag particles**. *J Virol* 2002, **76**:7544-7553.

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In an effort to examine the mechanisms underlying VLP-mediated induction of adaptive immune responses, the authors could show that HIV VLPs were capable of activating DCs *in vitro* resulting in increased expression of maturation markers and cytokine secretion. Besides, *in vivo* administration of VLPs equipped with the DC growth factor Flt3 triggered expansion of multiple types of immune cells including DCs, monocytes/macrophages as well as T and B cells thereby eliciting strong VLP-directed immune responses in mice.

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This interesting piece of work substantiates the proposed role of contaminating baculoviruses in VLP-mediated immune stimulation. The authors demonstrate that baculoviruses *per se* are capable of inducing strong humoral and CTL responses against a co-administered antigen by directly maturing DCs via stimulation of IFN-α secretion. The observation that adjuvant effects of VLPs were significantly compromised upon removal of baculovirus contaminations suggests to critically scrutinise the immunogenic properties of VLPs.

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