Particle-based delivery of HIV Envelopes

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Abstract

Purpose of review: A major focus in HIV vaccine research is the development of suitable antigens that elicit broadly neutralizing antibody responses targeting HIV's envelope protein (Env). Delivery of Env in a repetitive manner on particle-based carriers allows higher-avidity interactions and is therefore expected to efficiently engage B cells thus leading to affinity maturation that results in superior antibody responses characterized by improved breadth, potency and durability. This review summarizes current work that is evaluating diverse types of such particulate carriers for Env-delivery.
Recent findings: Various types of particle scaffolds are being investigated, encompassing Gag-derived virus-like particles, bacteria-derived proteins that self-assemble into symmetrical nanoparticles, as well as liposomes assembled from membrane components and recombinantly produced Env isoforms. Env-derived antigens from peptides over selected isolates to improved, stabilized next-generation designer Envs have been attached to such carriers. Immunological evaluation in animal models showed that these structures often elicit superior humoral immune responses.
Summary: The findings reviewed here emphasize the potential of particle-based delivery modalities to elicit better antibody responses. Together with advances in Env antigen design, these approaches may synergistically act together on the way to obtain vaccine candidates that potentially induce protective immune responses against HIV.

Key Points

- Particulate carriers for the presentation of Env-antigens are conceptually superior immunogens for the elicitation of high-quality B cell responses.
- A wide variety of scaffolds encompassing Gag-derived virus-like particles, synthetically assembled liposomes, and nanoparticles made from self-assembling proteins constitute a versatile toolbox for antigen delivery.

- Immunological evaluation in animal models confirms that Env-carrying particles often elicit better humoral immune responses such as improved serum neutralization capacity as compared to the analogous soluble Env antigens.
- Advances in Env antigen design and the production of particle carriers may converge into HIV vaccine candidates capable of eliciting protective immune responses.

Introduction

Virus-like particles (VLPs) are particulate structures that resemble viruses with the defining distinction that they cannot replicate because they do not carry viral genetic information [1–3]. As diverse as viruses are, so are the different types of VLPs. They are built at least from those viral proteins that are responsible for virus particle formation. In addition, they can contain other virus proteins like surface-bound envelope proteins. In general, two main types can be distinguished with regard to their architecture: VLPs that have a lipid membrane similar to enveloped viruses, and those that are proteinaceous aggregates similar to non-enveloped viruses [4].
Although not considered VLPs in the strict sense, there are also other artificial constructs that are functionally similar to VLPs but not made of viral components. Such structures are either artificially assembled liposomes in which viral proteins such as envelope-proteins are embedded, or structures consisting of aggregating proteins most often termed nanoparticles (NPs) [5].
VLPs, NPs and similar constructs are being explored as vaccine candidates because conceptually they have several immunologically beneficial properties. First, as they come close to the original virus in size and shape, VLPs are what our immune system has learned to deal with during evolution [6]. Second, uptake of a VLP into an antigen-presenting cell (APC) at once delivers many copies of each antigen thus possibly exceeding thresholds required for priming T cell responses [7]. Third, the repetitive nature of proteins on the surface can efficiently engage and cross-link B-cell-receptors by higher-avidity interactions. This initiates germinal center reactions, also supported by follicular helper T cells, during which somatic hypermutation takes place that leads to affinity maturation of the antibodies, increases in breadth, and the generation of long-lasting antibody responses [2,8]. Thus, particle-based vaccines take a position between highly-immunogenic live-attenuated virus-vaccines that – especially in the case of HIV – are often considered not to be safe enough, and subunit vaccines that are usually less immunogenic [9]. Finally, VLPs can also be used to deliver co-stimulatory molecules or to preferentially target dendritic cells [10].
The value of VLP vaccines is emphasized by the licensure of VLP-based vaccines, namely against hepatitis B virus [11] and human papillomaviruses [12], as well as hepatitis E virus in China [13]. Accordingly, particle-based approaches are heavily being investigated in the HIV vaccine field as well [14,15].

HIV Env as Antigen

The major focus in HIV vaccine development currently is the design of antigens derived from HIV's envelope protein (Env) capable of eliciting broadly neutralizing antibody (bnAb) responses, though other functional antibody and T cell responses might be important as well. Various Env-derived variants are being explored, ranging from peptides to native-like, soluble trimeric Env complexes [16*]. The latter are

generated by truncating the full-length Env (gp160) before the transmembrane domain, thus yielding the exterior part of Env (gp140). Especially Env-mimetics such as the SOSIP (SOS denoting an artificially introduced disulfide bond to prevent shedding, IP denoting an Ile-to-Pro mutation that stabilizes the pre-fusion state) [17] or NFL ("native flexibly linked", where a flexible linker replaces the furin cleavage motif) design [18] adopt such native-like conformations (see also the article by M. Ramirez in this issue). Although autologous tier 2 neutralizing responses can be elicited in animal models by using such Env-mimetics or Envs from specially selected virus strains [19*,20], there are several rationals for delivering Env in a particulate configuration.

Besides the generally beneficial VLP properties described above that are expected to contribute to increasing the strength, breadth and durability of the antibody responses [16*], there are several aspects pertaining specially to Env. First, gp140 is rather instable and therefore prone to exhibit epitopes that elicit non-neutralizing antibody (nnAb) responses that may distract development of the desired bnAbs. Thus, besides stabilization via protein modifications as in SOSIPs or NFLs, linking Env to aggregating scaffolds can stabilize the trimers. Moreover, Env seems to be generally more stable in a membrane environment [21]. Second, the membrane-proximal external region (MPER) of Env is one site of vulnerability with respect to neutralizing antibodies and the epitopes of some MPER-directed bnAbs also partially comprise membrane regions [22*] (see also the article by W. Weissenhorn in this issue). Moreover, the hydrophobic MPER is often removed from gp140 to avoid aggregation of soluble trimers thus principally preventing the generation of MPER-specific antibodies with these antigens [23]. Third, the bottom side of the soluble trimers may represent a neo-epitope that might elicit nnAbs as demonstrated in mice [24] and that would be shielded in a particle-bound state.

Thus, it is widely believed that epitopes of an antigen as complex as Env are best displayed on VLPs [25]. Consequently, numerous approaches for the delivery of Env on the surface of particles as HIV vaccine candidates are currently being pursued (Fig. 1).

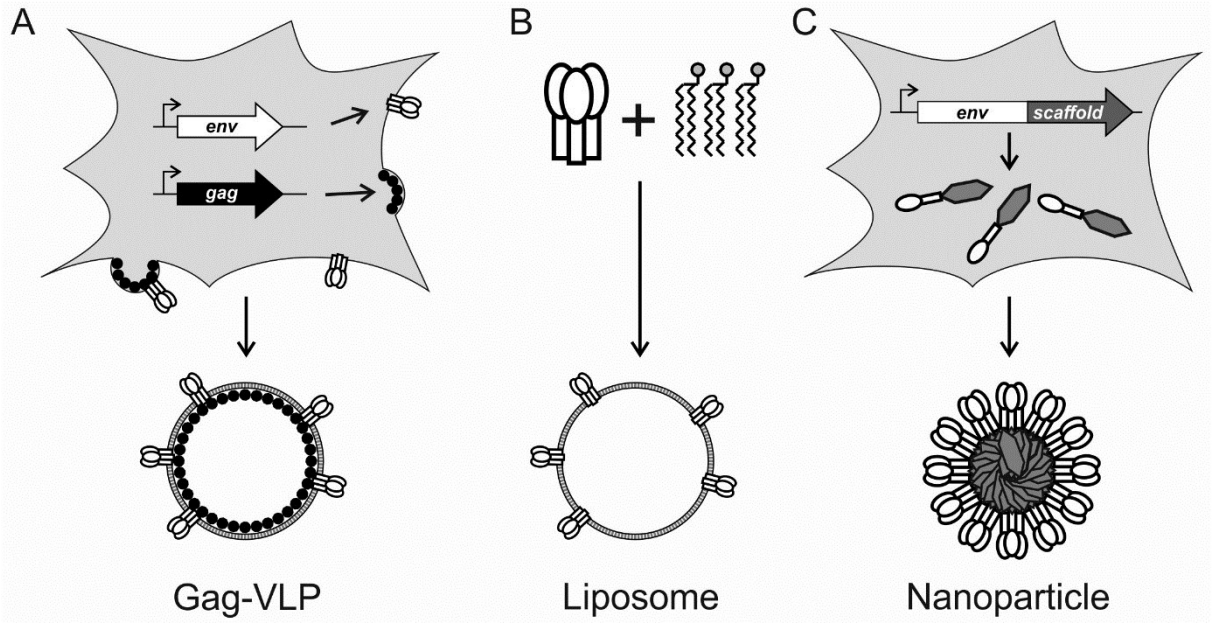

Figure 1) Generation of Env-carrying particles

A) Expression of *gag* and *env* genes in mammalian cells leads to budding of Gag-VLPs in which Env molecules from the cell-surface can get incorporated. **B)** Liposomes can artificially be assembled from phospholipid components and recombinantly produced, purified Env molecules. **C)** Expression of scaffold proteins that self-assemble into aggregates leads to the formation of nanoparticles. Env variants can be attached via genetic fusion as indicated, or chemically attached post production (not shown).

Gag-derived VLPs

Expression of HIV's group-specific antigen (Gag) in mammalian cells readily leads to the formation of Gag-VLPs. The Gag protein alone is necessary and sufficient to drive HIV particle formation. Gag proteins from all retroviruses form VLPs but have distinct morphological features [26]. Even Gag genes from human endogenous retroviruses can get expressed under certain circumstances and form VLPs [27,28].
Gag is an important anti-HIV antigen by itself and has been used in many vaccine approaches to elicit CTL responses [29]. Therefore, and also due to the arguments provided above, Gag-VLPs have been used already very early as a platform to present Env [30] and the immunogenicity of such particles has subsequently been confirmed [31]. Another advantage is that the Gag-component can provide intrastructural help [32,33*,34] where the uptake of an Env-carrying Gag-VLP into a respective Env-specific B cell leads to presentation of Gag-derived peptides on MHC class II molecules. Thus, the Env-specific B cell can receive T cell help from Gag-specific T cells, leading to better antibody responses (see also the article by K. Überla in this issue).

Env-presenting Gag-VLPs

Interestingly, there are only few (7 – 14) Env trimer complexes on HIV virions [35] and this low density is replicated on Gag-VLPs. How Env is incorporated into Gag-VLPs is not quite clear and several mechanisms are being discussed [36]. By removing or exchanging the cytplasmic tail and the transmembrane domain, Env incorporation can, however, be increased [37]. Similary, Vzorov et al. [38] achieved an increase in Env-incorporation that also translated into higher neutralization activity of sera from guinea pigs immunized with a DNA-prime, VLP-boost regimen.
A drawback of Env-presentation on Gag-VLPs is that not all Envs may have a native conformation after VLPs are released from the producer cells. Tong et al. developed an interesting solution to mitigate this. They treated Gag-VLPs carrying the JR-FL Env with a mixture of proteases that destroys non-functional Env molecules while those with native conformation, as defined by the binding profile of an appropriate set of bnAbs and nnAbs, remain intact [21]. Immunogenicity analysis of so-called "trimer VLPs" in rabbits [39] yielded high neutralizing activity in some animals that was mainly targeted towards sites where certain glycans are naturally absent in the vaccine strain. In contrast, immunogenicity analyses of SIVmac239 VLPs that were not protease-treated, carrying a non-stabilized gp160 in comparison to a mixture of the corresponding soluble gp140 and empty Gag particles showed a non-significant trend that the soluble Env was superior in rhesus macaques [40], possibly due to the presence of non-functional Env on the VLPs.

These studies confirm that VLPs are good carriers to deliver natively structured Env trimers but that selection or special design of a suitable Env will be necessary to achieve the desired broad tier 2 neutralization activity.

Env-carrying liposomes

Liposomes are artificial membrane assemblies that resemble empty enveloped viruses. Ingale et al. [41**] coupled His-tagged Env variants to phospholipids complexed with Ni^{2+} that were inserted into such liposomes resulting in a high density of Env on the surface. The vehicles led to enhanced B cell activation *ex vivo* and improved germinal center reactions in mice as compared to the respective soluble protein. This confirms the notion that repetitive antigens are superior stimulants of the processes leading to affinity matured antibodies. In another study, lipid nanocapsules (considered more stable than conventional liposomes) were generated that consist of several covalently coupled sheets of lipid bilayers [42] to which naturally stable trimeric gp140 proteins of isolate 92UG037.8 were attached [43]. These nanocapsules also elicited higher Env-specific antibody titers in mice that were also broader reactive to a set of linear peptide epitopes.
A special form of liposomes are so-called proteoliposome nanoparticles (PLN) that are generated by first attaching chemically cross-linked, and Flag-tagged gp160 Env molecules purified from native virions via a tag-specific antibody to 200 nm sized magnetic beads [44*]. Subsequently, lipid components are added that form a membrane around the particle in which the Env molecules get embedded [45]. Immunization of rabbits with these PLNs was compared to immunizations with Gag-VLPs, as well as purified, cross-linked Env-trimers in an appropriate detergent. The reference regimens only elicited tier 1a neutralizing sera, whereas some sera from PLN-immunized rabbits showed tier 1b and autologous tier 2 neutralization despite modest titers in all groups.

Env-carrying nanoparticles

An alternative to lipid membrane enclosed vesicles are nanoparticles that mostly are formed from protein scaffolds that self-assemble into symmetrical aggregates, thus being capable of presenting Env in a highly-ordered, repetitive arrangement [5]. Several such scaffolds – often derived from bacteria – have been investigated so far (Fig. 2). For instance, in analogy to an influenza vaccine candidate based on ferritin (FR) nanoparticles [46,47], Zhou et al. [48] attached the glycan V3-supersite to this scaffold. The nanoparticles were antigenic and the apparent affinity of the respective antibodies was improved. Sliepen et al. assessed the immunogenicity of BG505 SOSIP.664 trimers presented on such FR-particles [49*]. The ferritin protein derived from *H. pylori* self-assembles into a 24-mer particle, thus the fused Env-protein can stoichiometrically fold into eight Env-trimers. Morerover, FR is chemically and thermally very stable, thus being a valuable scaffold. As shown by negative-stain electron microscopy, Env spikes did protrude from the nanoparticles. In mice, the FR-particles elicited significantly higher Env-specific antibody titers, whereas in rabbits the responses were only slightly higher than those to the soluble protein, but 4 of 5 rabbits of the FR group developed autologous tier 2 neutralizing responses.

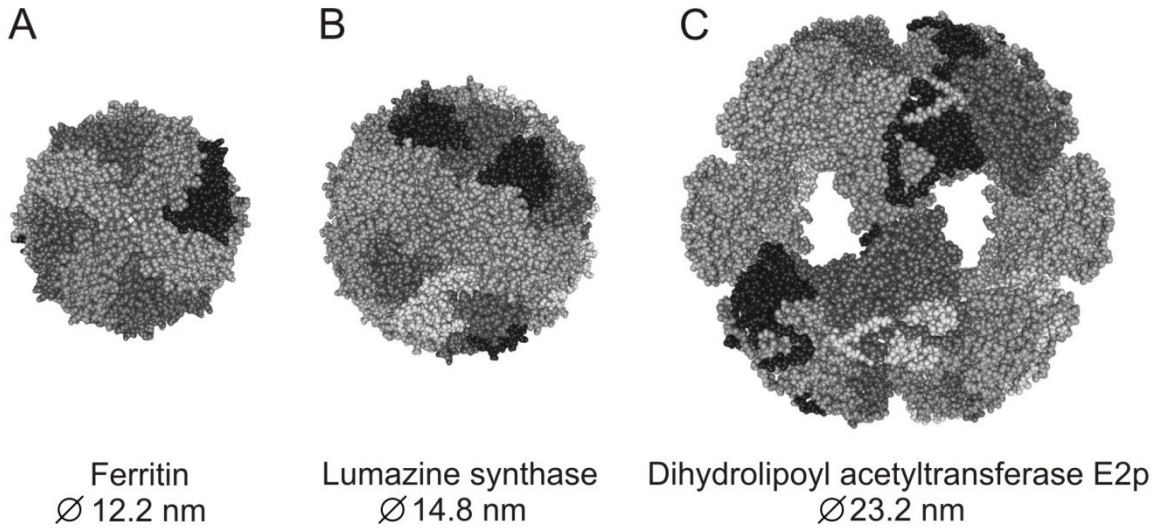

Figure 2) Structures of selected nanoparticle scaffolds
A) Ferritin from *H. pylori* forms a 24-mer aggregate (drawn from pdb structure 3BVL). **B)** Lumazine synthase from *Aquifex aeolicus* forms 60-mer aggregates (pdb 1NQW). **C)** 60 subunits of the dihydrolipoyl acetyltransferase E2p of *Bacillus* assemble into a hollow dodecahedron made of trimeric substructures (pdb 1B5S). Approximately drawn to scale.

Jardine et al. [50] generated an Env variant termed "eOD-GT6" comprising an engineered outer domain of monomeric gp120 that binds to the predicted germline BCR precursor of the VRC01 antibody. They fused this variant to lumazine synthase (LS, an enzyme involved in riboflavin synthesis) from *Aquifex aeolicus* (a thermophilic baterium), a protein that self-assembles into a 60-mer aggregate. Heterologous expression in mammalian cells leads to secretion via the secretory pathway into the culture supernatant from which the assembled nanoparticles can be purified using techniques commonly used also to purify the soluble Env trimers. The LS particles readily activated B cell lines expressing the germline or mature VRC01 BCR. In contrast, a monomeric eOD-GT6 variant was non-stimulatory, presumably due to inefficient BCR-crosslinking. Thus, the polyvalent nature of the LS particles leads to an avidity gain that compensates for the otherwise low affinity interaction. To confirm this *in vivo*, the authors generated a knock-in mouse harboring the inferred germline-reverted VRC01 gH-chain [51**]. Immunization with a further improved variant, LS-eOD-GT8 [52] indeed led to activation of such B cells. Antibodies directed towards the CD4-binding site (CD4bs) were elicited with the nanoparticle being more potent in these knock-in mice. Boosting these responses with a sequential antigen led to antibodies that could neutralize a near-native isolate [53*]. Similarly, eOD-GT8 also led to the activation of B cells in knock-in mice harboring the CD4bs-directed bnAb 3BNC60 heavy chain [54]. Recently, Sok et al. furthermore tested the eOD-GT8 antigen in so-called Kymab-mice that express the whole human variable Ig gene repertoire [55] and found that nearly 30 % of these mice mounted VRC01-class antibody responses despite extremely low frequencies of the corresponding precursor B cells [56]. Thus, regimens involving sequential immunizations starting with designer antigens activating germline B cells and followed by antigens that are closer to the native Env proteins, are a promising approach to guide the development of antibody responses into a desired direction (see article by D. Peterhoff in this issue). Importantly, eOD-GT8-reactive B cells could also be detected among

peripheral blood mononuclear cells from HIV-negative donors [52] which is an important prerequisite for such an approach.
In another study, the generation of several nanoparticle scaffolds presenting different Env configurations was systematically compared [57*]. FR-bound V1V2 peptides had a native-like, trimeric conformation, as they reacted with select bnAbs like PG9. Similary, a ferritin-gp120-fusion gave rise to well-formed particles with trimer-like gp120 Env molecules displayed on the surface that were antigenically superior to soluble gp120, emphasizing the gain in avidity obtained by presentation on particles. LS-gp120-particles, however, could not be obtained. The dihydrolipoyl acetyltransferase from a Bacillus species, E2p, forms a hollow dodecahedron made up of 60 subunits. Although E2p-particles with gp120 did not show gp120 trimers in EM analyses, probing with bnAbs confirmed the presence of trimeric gp120. Finally, use of a redesigned gp140 trimer [58] led to well-ordered Env-carrying E2p-particles that again exhibited an improved antigenicity profile as compared to the soluble protein. It will be interesting to see whether these nanoparticles also exhibit improved immunogenicity.
Overall, these studies make clear that not all particle platforms are suitable for the presentation of any antigen. Rather it is important to select the right platform for the respective application.

Vectored delivery of VLP vaccines

Vaccination regimens involving nucleic acid or viral vector delivery systems can in principle be used to deliver VLP vaccines that assemble *in vivo*. For instance, production of Gag-VLPs has been shown for a recombinant vaccinia virus expressing a Gag variant and gp140 [59]. Of course, Env is not incoporated into these VLPs in this case, however, the aim here was to elicit potent T cell responses. It could be confirmed in rhesus macaques that very strong and polyfunctional responses were indeed obtained with a DNA prime and vaccinia virus boost regimen [60]. Yet, employment of an Env-variant harboring a transmembrane domain would in principle allow the generation of Env-carrying Gag-VLPs. Such constructs were used by Iyer et al., who assessed a vaccination regimen in rhesus macaques that included an MVA vaccinia virus expressing SIVmac239-derived Gag and Env [61]. A subgroup eventually received a boost with recombinantly produced gp160-carrying VLPs that were adjuvanted with several TLR agonists. Humoral Env-specific responses were readily obtained and strongly boostered by the VLP injection (titers increased on average 20-fold), as well as – expectedly – Gag-specific responses. Mapping studies with linear peptides showed an increase in breadth after the VLP boost. However, challenge with SIVmac239 showed no significant differences, although there was a slight delay regarding the number of challenges until infection occurred for the VLP-group.
Andersson et al. generated recombinant adenoviruses that express a SIVmac239 Gag-protein linked to an M-group consensus Env via a 2a-peptide, so that during translation two separate proteins are generated from the same mRNA [62]. Analysis of transduced cell lines confirmed that Env-carrying VLPs are indeed generated. In Balb/c mice, the Ad-vectors elicited antibody responses and most of the sera were also capable of neutralizing tier 1 pseudoviruses. Comparison of full-length Env with a variant truncated after the transmembrane domain confirmed that the cytoplasmic tail can affect the conformation of the extracellular part of Env [63] and thus its antigenicity [64]. This again emphasizes that the quality of the antibody responses

that is obtained is not only dependent on the way of delivery, but also on the design and configuration of the Env antigen itself. As the Env proteins are produced *in vivo* in settings of vector delivery, it is necessary that Env variants are used that nearly exclusively form native-like trimers, as the common purification procedures to remove monomers or uncleaved Env molecules are of course only applicable to recombinantly produced proteins.

Conclusion

Several important advances have been made within the last few years that now allow the elicitation of autologous tier 2 neutralizing antibody responses towards HIV Env. This is mainly due to improvements of Env conformations, but, as summarized here, the higher-level configuration of the antigens on particle carriers also crucially contributes to the magnitude and quality of the corresponding antibody responses. Further advances in Env design on the one hand, and in VLP and nanoparticle production on the other hand, will hopefully converge synergistically into superior vaccine candidates capable of eliciting broader, cross-clade neutralization responses. Finally, it will be very interesting to see how well particle-based HIV vaccine candidates perform in clinical trials, and whether the benefits that VLPs and NPs have in animal models are also valid in humans.

Conflicts on Interest

none

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