



Rotavirus VP6: involvement in immunogenicity, adjuvant activity, and use as a vector for heterologous peptides, drug delivery, and production of nano-biomaterials

Zabihollah Shoja¹ · Somayeh Jalilvand² · Tayebeh Latifi² · Farzin Roohvand¹

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Abstract

The first-generation, live attenuated rotavirus (RV) vaccines, such as RotaTaq and Rotarix, were successful in reducing the number of RV-induced acute gastroenteritis (AGE) and child deaths globally. However, the low efficacy of these first-generation oral vaccines, coupled with safety concerns, required development of improved RV vaccines. The highly conserved structural protein VP6 is highly immunogenic, and it can generate self-assembled nano-sized structures, including tubes and spheres (virus-like particles; VLPs). Amongst the RV proteins, only VP6 shows these features. Interestingly, VP6-assembled structures, in addition to being highly immunogenic, have several other useful characteristics that could allow them to be used as adjuvants, immunological carriers, and drug-delivery vehicles as well as acting a scaffold for production of valuable nano-biomaterials. This review provides an overview of the self-assembled nano-sized structures of VP6-tubes/VLPs and their various functions.

Introduction

Rotavirus (RV) virions are triple-layered particles with icosahedral symmetry, enclosing 11 segments of double-stranded (ds) RNA, the viral RNA-dependent RNA polymerase (VP1), and the capping enzyme (VP3) [14, 39, 45]. RVs were first identified in animals [1, 75], and later in humans [12, 13, 48, 63]. They are prominent causative agents of acute gastroenteritis (AGE) in children less than 5 years of age worldwide [93, 94, 112] and were responsible for around 500,000 deaths each year, prior to the beginning of global vaccination programs [112]. However, after the introduction of RV vaccines, the number of RV deaths decreased to 215,000 in 2013 [113] and then to 128,500 in 2016 [115],

globally. The vaccines that have been introduced include live attenuated oral vaccines, which are used at both the global (RotaTaq and Rotarix) [7, 8, 19, 20, 106, 119, 120] and national (RotaVac, Rotavin-M1, ROTASIL, and Lanzhou lamb RV vaccines) [7–10, 19, 20, 59] levels. Despite the success of live attenuated oral vaccines in reducing the number of RV deaths worldwide, several concerns, such as efficacy, safety, and cost, have impeded their development, and novel alternative approaches to producing RV vaccines are being considered.

Among the RV proteins, the highly conserved structural protein VP6 was the first RV antigen (Ag) to be used for classification of RV strains. Accordingly, 10 groups (A–J) and two new tentative groups, K (for RVC-like group) and L (for RVH-like group) [6, 58, 62, 82, 87], have been identified based on serological cross-reaction to the VP6 protein (group Ag). In addition, further VP6 classification into four subgroups (SGI, II, I + II, and non-I, non-II) based on reactivity with one, both, or neither of the monoclonal antibodies (mAbs) 255/60 and 631/9 has been proposed [52, 73]. However, other classification systems based on the genotypes of the outer capsid proteins VP7 and VP4, the glycoprotein 36G, and the protease-sensitive protein 51P have also been described [58, 69, 79, 81]. Furthermore, a whole-genome-based RV classification system has also been introduced that

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Zabihollah Shoja and Somayeh Jalilvand have contributed equally as first author.

✉ Zabihollah Shoja
z_shoja@pasteur.ac.ir; zshoja@razi.tums.ac.ir

¹ Department of Virology, Pasteur Institute of Iran, Tehran, Iran

² Department of Virology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran

defines the genotypes of all 11 genomic RNA segments [79, 80].

Besides being a highly immunogenic protein containing dominant antigenic epitopes, VP6 is also the most abundant viral protein and is capable of self-assembling into complex structures when expressed in isolation under defined conditions. These structures include trimeric, spherical, and tubular forms and sheet structures. Structural proteins from several viruses such as hepatitis B virus (HBV), human papilloma virus (HPV), human immunodeficiency virus (HIV), adeno-associated virus (AAV), hepatitis C virus (HCV), norovirus (NoV), human influenza virus, respiratory syncytial virus (RSV), coronaviruses, and bacteriophages have also been shown to be capable of producing immunogenic VLPs that can be used for vaccine development [88, 100]. However, VP6 is one of only a limited number of structured viral protein assemblies that are able to be used as carrier proteins with strong adjuvant activity. Moreover, VP6 is one of the very few immune-carrier/adjuvant systems that are capable of producing tubular forms and sheet structures. One well-known example of a viral carrier protein is the highly immunogenic hepatitis B core antigen (HBcAg), which is used as a carrier for heterologous Ags/epitopes to enhance antibody production and T-cell responses directed to heterologous antigens [105]. However, despite the powerful adjuvant activity of HBcAg VLPs, they do not make tubular forms or sheet structures like VP6.

The first biochemical analyses of the native oligomeric structure of VP6 were performed using infected cells or virus particles, selectively removing VP6 from double-layered particles (DLPs; VP2/6) [11, 51]. In parallel, several other researchers attempting heterologous expression of RV VP6 also reported the production of this protein in its native oligomeric state [2, 17, 37, 38, 67, 72, 89, 90, 126, 127] and suggested the use of nano-tubular (tube)/nano-spherical (VLP) structures of VP6 as an immunological carrier for heterologous and homologous Ags [49, 102, 111] as well as a drug delivery system [126]. Of note, these structures were shown to react with VP6-specific mAbs, indicating that their native immunoreactive determinants were conserved [44]. It has also been shown that self-assembly of VP6 into nano-scale structures can create multifunctional scaffolds for construction of organized nanoparticle-based biomaterials with novel functions and characteristics [99]. Therefore, based on its strong immunogenicity, conserved characteristics, and ability to self-assemble, VP6 is being considered as both an antigen and a platform to develop alternative non-living RV vaccine candidates, as well as an organized nanoparticle-based structure for biomedical applications.

We have previously reviewed several promising VP6-based vaccine platforms (VP6 DNA vaccine, VP6 recombinant protein vaccine, and VP6-VLPs vaccine) [3, 60] that were able to induce heterologous cross-protective

immunity to RV in animal models. In the present article, we provide an updated overview of potential applications of VP6 tubes/VLPs as an adjuvant, immunological carrier, and drug delivery tool and their use in the design of a new generation of vaccines and as a scaffold for generation of nano-biomaterials.

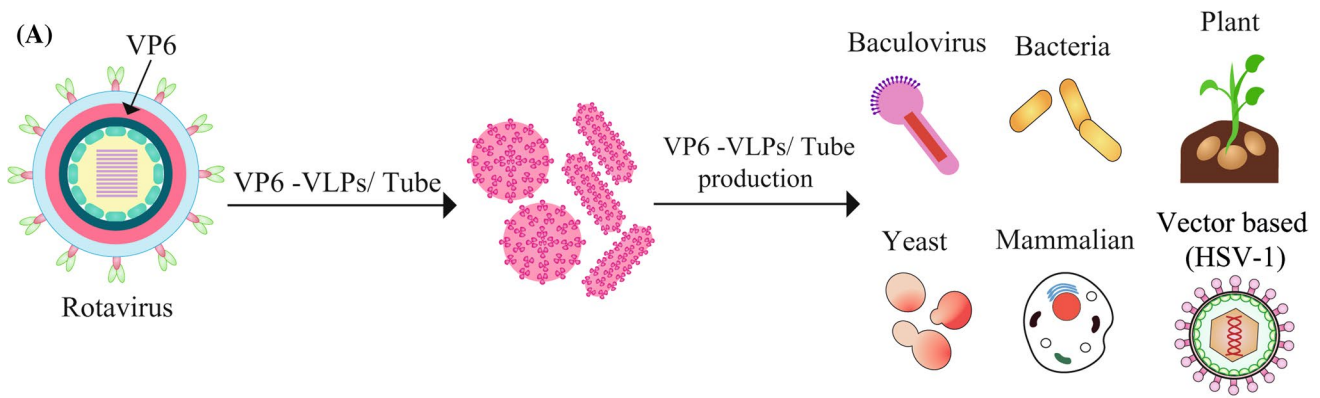
VP6 expression and formation of structured VP6 tubes/VLPs

Various expression systems (Fig. 1A), from the prokaryotic *E. coli* [2, 72, 126] to eukaryotes such as yeast (including the baker's yeast *Saccharomyces cerevisiae* and the methylotrophic yeasts *Pichia pastoris* and *Hansenula polymorpha*), baculovirus-silkworm systems [17], insect cell/baculovirus (IC-BV) systems [38, 67], mammalian cells [38], herpes simplex virus 1 (HSV-1)-based vectors [90], and plant cells [37, 89, 127] have been used to produce multimeric VP6 structures that morphologically resemble VP2- and VP6-containing VLPs, as well as tubes.

The assembly of VP6 into different structural forms depends on the pH, ionic strength, and concentrations of the divalent cations Ca^{+2} and Mg^{+2} [68, 101]. Spherical multimeric structures are formed in the pH range of 3.5–5.5, whereas large and small tubular multimeric structures are formed in the pH range of 5.5–7 and above 7, respectively [68, 86]. In contrast, structured protein assemblies of VP6 are disassembled at Ca^{+2} concentrations above 10 mM [68, 86] and at increased oxidant concentrations during the stages of the particle formation [25]. The formation of VP6 nanotubes *in vitro* was first reported in 1987 [101], but recently, a simple and efficient ultrafiltration method to purify VP6 tubes/VLPs *in vitro* was developed [67]. More recently, an easy and reproducible method to produce VP6 nanotubes from VP6 monomers was introduced that not only offers a novel approach to producing VP6 tubes *in vitro* but also makes it possible to modulate the length of the tubes [104]. Progress in the production/purification and development of various VP6 tubes/VLPs has provided the opportunity to investigate their potential application as immunogens, adjuvants, delivery tools, and nano-biomaterials (Table 1).

VP6 immunogenicity

Various RV VP6 Ag-encoding vaccine modalities, including DNA vaccines [3, 26–28, 36, 56, 57, 60, 74, 121, 123, 125, 128], subunit vaccines (harboring recombinant VP6 protein) [2, 3, 29–35, 40, 46, 60, 66, 83–85, 107, 116, 117, 121, 124, 127], and self-assembled structures [3, 15, 16, 18, 44, 60, 64, 76–78, 95, 108, 110, 121] have been reported to induce an immune response and/or protection



(B) VP6 -Tube/VLPs

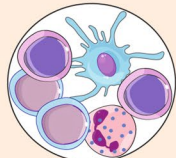
<p>Adjuvant</p> <ul style="list-style-type: none"> ● VP-6tubes/VLPs can act as an adjuvant and enhance prominent Ab and T cell immune responses against foreign Ags ● Co-administration of VP-6VLPs with norovirus P particle indicate the adjuvant effect of VP-6VLPs 	<p>Immunogenicity and Protection</p> <ul style="list-style-type: none"> ● Induction of robust, long lasting, high avidity IgG responses against RV ● Induction of Th1/ Th2 and high levels of cross-reactive serum IgGs against different RV strains, as well as mucosal IgG, IgA Abs and high level of IFN-γ production ● Generating CD+4 T cell subsets and induction of antiviral cytokine IL4, IL17 and IFN-γ 	<p>Nano-Biomaterials</p> <ul style="list-style-type: none"> ● Act as a scaffold to allow charge transfer processes ● Create bio-electrochemical interfaces ● Act as a scaffold for the synthesis of the metallic nanoparticles such as silver, gold, platinum, and palladium
<p>Immuno/carriers</p> <ul style="list-style-type: none"> ● Using of RV VP6 structured assembles for chimeric Ag delivery in paramyxovirus, poliovirus, RV, influenza A virus, and SARS-CoV2 ● Showing of high titers of Abs and strong T cell immune responses against both VP6 and the inserted Ag 	<p>Drug Delivery Tools</p> <ul style="list-style-type: none"> ● VP6 fused to SUMO maintain their nanotubes/VLPs structure in the gastrointestinal tracts when administered orally 	

Fig. 1 The RV protein VP6 is capable of self-assembling into nano-sized spherical and tubular forms when expressed in isolation under defined conditions. (A) Different expression systems from prokaryotic *E. coli* [2, 72, 126] to eukaryotic systems such as yeast [17], insect cell/baculovirus systems (IC-BV) [38, 67], mammalian cells [38], HSV-1 [90], and plant cells [37, 89, 127] have been used suc-

cessfully to produce assembled structures of VP6 tubes/VLPs. (B) Applications of VP6 tubes/VLPs, as vaccines (immunogenicity and protection) [15, 44, 54, 55, 64–66, 70, 95, 108], adjuvants [15, 16, 55, 76–78, 108–110], immunological carriers [49, 53, 92, 96, 102, 111], drug delivery tools [5, 91, 122, 126], and nano-biomaterials [5, 23, 24, 41, 42, 50, 91, 122, 126]

in animal models. The results of these animal immunization studies have indicated that, even in the absence of neutralizing Abs, immune responses to VP6 alone might be protective. It is known that neutralizing epitopes are only present on the VP4 and VP7 proteins of RV. Therefore, the observed protection of animals immunized with VP6 preparations lacking VP4 and VP7 proteins indicates that neutralizing Abs (directed against VP4 and VP7) might not be needed for protection against RV infection [3, 60]. Similarly, there have been several reports showing

that non-neutralizing Abs are capable of clearing RV infection, suggesting the occurrence of intracellular inhibition of RV infection by IgA [4, 21, 47] and IgG [22] following transcytosis. Interestingly, among the various VP6 preparations, self-assembled VP6 structures such as VP6 tubes/VLPs have been reported to be the strongest immunogens, not only inducing a stronger immune response and a higher level of immunogenicity and protection [95] but also exhibiting adjuvant and immunological carrier properties [15].

Table 1 Summary of the reports on the multiple functions of RV VP6 tubes/VLPs

Property/application	Reports	References
(i) VP6 tube/VLP immunogenicity	<p>VP6 tubes/VLPs have potential as non-living RV vaccines</p> <p>VP6 tubes protect mice more efficiently from RV challenge than trimers or DLP2/6, suggesting their superiority for vaccine formulations</p> <p>A combination of norovirus GII-4/GI-3 VLPs and RV VP6 tubes maintained the immunogenicity of the VP6 tube and the other Ags, suggesting the possibility of developing mixed vaccines against both pathogens</p> <p>VP6-tube-specific Abs in the gut of immunized animals might act as a first line of defense to inhibit infectivity of different RV strains, suggesting a significant role of mucosal immunity and VP6-specific IgA in inhibition of RV replication and heterotypic protection against RV infection</p> <p>The generation of cellular immune responses (IFN-γ, IL-4, and pro-inflammatory cytokine IL-17) implies that VP6 tubes provide protection against RV infection by other mechanisms besides mucosal IgA induction</p>	[15, 44, 54, 55, 64–66, 70, 95, 108]
(ii) The adjuvant role of RV VP6 tubes/VLPs	<p>A priming effect of RV VP6 immunization to enhance the elicitation of the neutralizing antibodies against heterotypic RV VP4 or VP7 proteins was documented</p> <p>Combined vaccines (norovirus GII-4, GI-3 VLPs, and VP6 tubes/VLPs) induced higher titers of cross-reactive Abs against norovirus genotypes than non-combined vaccines, indicating the adjuvant effect of RV VP6 on norovirus GII.4 or GI.3 VLPs</p> <p>The adjuvant effect of VP6 tubes on co-administered norovirus VLPs (as a mixture) is strictly dependent on co-localization of VP6 with norovirus VLPs, which was further supported by showing the enhanced uptake of norovirus VLPs into the APCs</p> <p>VP6 tubes/VLPs exerted similar dose-dependent adjuvant effects on norovirus-specific Ab responses</p> <p>The particulate nature and size of the co-administered Ag might also affect the adjuvant effect of the VP6-VLPs</p> <p>Similar adjuvant effects were observed using plant-derived VP6 tubes co-administered with plant-derived norovirus VLPs, indicating the independence of this adjuvant characteristic on the system used for vaccine production</p>	[15, 16, 55, 76–78, 108–110]
(iii) VP6 tubes/VLPs as immunological carriers	<p>RV VP6 structured protein assemblies were used successfully for chimeric Ag delivery. Some examples are insertion of (i) a peptide derived from the RV VP4 protein, (ii) a 14-amino-acid peptide derived from simian paramyxovirus 5 (V5 epitope), (iii) three epitopes of poliovirus type 1, (iv) three epitopes derived from the VP4 of RV, (v) two peptides (23 and 140 amino acids) derived from the M2 and HA genes of influenza A virus, and (vi) the receptor-binding domain (RBD) of the SARS-CoV-2 spike protein (S) to the surface loops or N-terminus of VP6</p>	[49, 53, 92, 96, 102, 111]
(iv) Drug delivery	<p>Biocompatible and biodegradable RV VP6 structured protein assemblies in the form of nanotubes/VLPs are capable of encapsulating therapeutic reagents for drug delivery and have been used successfully for treatment of diseases such as cancer.</p>	[5, 91, 122, 126]
(v) Nano-biomaterials	<p>The VP6 tubes/VLPs form multifunctional scaffolds that can be used to create bio-electrochemical interfaces for construction of organized metallic nano-biomaterials (silver, gold, platinum), and palladium (Pd)</p>	[5, 23, 24, 41, 42, 50, 91, 122, 126]

VP6 tube/VLP immunogenicity and protective measures

Due to their superior immunogenicity compared to other VP6 preparations, VP6 tubes/VLPs have been selected for use in VP6-based RV vaccine candidates. Despite the challenges to efficient VLP generation on a large scale in insect cells, the establishment of IC-BV expression systems has been given high priority for production of VP6 tubes/VLPs. Indeed, in the first studies in 1987 [44], a recombinant baculovirus encoding the VP6 gene of strain SA11 was used to express VP6 proteins capable of assembling into tubular structures while maintaining their native antigenic determinants and oligomeric structure [44], and several studies demonstrated the immunogenicity of these insect-cell-derived VP6 tubes/VLPs. In this context, immunization of BALB/c mice with a combination of norovirus GII-4 VLPs and RV VP6 tubes resulted in a robust systemic cross-reactive and cross-blocking antibody response to both norovirus and RV [15], indicating sufficient immunogenicity of both Ags in the combined preparation without any dominance of one over the other. In line with this finding, a trivalent combination vaccine containing norovirus GII-4 and GI-3 VLPs and RV VP6 tubes was also formulated by the same group of the researchers [108]. Immunization of mice with this trivalent vaccine demonstrated the ability of VP6 tubes to induce a robust, long-lasting, high-avidity IgG responses against various RV strains [108]. In still another study by the same group, the immunogenicity of the assembled VP6 tubes and double-layered (dl) 2/6-VLPs produced by coexpression of VP2 and VP6 was investigated [64]. The results indicated that this vaccine formulation induced a balanced Th1/Th2-type immune response with high levels of cross-reactive serum IgG against different RV strains, as well as mucosal IgG and IgA Abs, and cellular immune responses with high levels of IFN- γ production [64]. Of particular note, the results of these and further studies indicated that mucosal VP6-specific Abs against VP6 tubes, which are found in the gut of immunized animals, might act as a first line of defense to inhibit infection by different RV strains *in vitro* and *in vivo* [64, 65, 108]. Similarly, other immunization studies in which VP6 assemblies were administered via the intramuscular or intranasal route in BALB/c mice indicated that serum VP6-specific IgA titers correlated with at least 65% protection against RV infection regardless of the delivery route [66]. These results suggested a significant role of mucosal immunity and VP6-specific IgA in inhibition of RV replication and heterotypic protection against RV infection. It should be noted, however, that immunization of BALB/c mice with VP6 tubes via the intradermal or intranasal route is also capable of

generating diverse CD4⁺ T cell subsets and induction of the antiviral cytokine IFN- γ , interleukin-4 (IL-4), and the pro-inflammatory cytokine IL-17 [54]. The induction of such diverse cellular immune responses might imply that immunization with VP6 tubes provides protection against RV infection by other mechanisms besides intracellular inhibition by VP6-specific IgA and IgG.

In an attempt to assess the immunogenicity of the various assemblies of RV VP6 in vaccine formulations, the immune responses elicited by VP6 tubes, dl2/6-VLPs, and trimers were compared [95], and the results indicated that immunization of mice with one dose of VP6 tubes induced the highest IgG titers and provided a level of protection against RV infection similar to that of two doses of either dl2/6-VLPs or trimers, suggesting the superiority of RV VP6 tubes for vaccine formulations [95].

In addition to the baculovirus expression system, *E. coli* has also been used to produce various RV VP6 assemblies [18, 70–72]. The morphology of the *E. coli*-derived VP6 tubes/VLPs and trimers was verified using transmission electron microscopy (TEM) and atomic force microscopy (AFM) [18, 61, 70–72, 118]. Interestingly, the efficacy of the *E. coli*-derived VP6-VLPs or trimers against RV infection was significantly lower than that of *E. coli*-derived DLP2/6 [70]. In addition to baculovirus and *E. coli* expression systems, mammalian cells [38] and HSV-1-based vectors [90] as well as plant cells [37, 89, 127] have also been shown to have the capacity to produce self-assembled RV VP6 structures to induce protective immune responses against virus infection [78, 90].

Overall, among the different RV VP6 preparations, VP6 tubes/VLPs might have the highest potential as a non-living RV vaccine platform due to their strong immunogenicity and maintenance of conserved epitopes.

The adjuvant role of RV VP6 tubes/VLPs

Several studies have demonstrated the ability of RV VP6 tubes/VLPs to enhance immune responses against co-administered homologous/heterologous antigens (Fig. 1B). The priming effect of immunization with the RV VP6 protein to enhance the generation of neutralizing Abs against heterotypic RV VP4 or VP7 proteins has been reported [43]. Several years later, in the early 2010s, the adjuvant effect of VP6 tubes/VLPs on a co-administered heterologous antigen (norovirus GI-3 protein), enhancing the induction of GI-3-specific cross-reactive Abs in the immunized mice, was shown [15]. In still another recent study, the adjuvant effect of VP6 tubes in mice immunized with suboptimal doses of norovirus GII.4 or GI.3 VLPs, either alone or in combination with VP6 tubes, was investigated [16]. The results of that study indicated that suboptimal

doses of the norovirus VLPs alone did not induce substantial anti-norovirus Abs, but the combined vaccine induced considerable titers of cross-reactive Abs against both norovirus genotypes, indicating the adjuvant effect of RV VP6 on norovirus GII.4 or GI.3 VLPs [16]. It was later shown that the adjuvant effect of VP6 tubes on co-administered norovirus VLPs (as a mixture), compared to the separate administration of two antigens, is strictly dependent on co-localization of the VP6 with norovirus VLPs [77]. This finding was further supported by showing enhanced uptake of norovirus VLPs into antigen-presenting cells (APCs) [103] when mixed with VP6 tubes [76]. This process might induce the production of several cytokines/chemokines (tumor necrosis factor [TNF]- α , IL-6, IL-1, and granulocyte macrophage colony-stimulating factor [GM-CSF]), indicating the potential adjuvant effect of VP6 on norovirus-specific T-cell immunity [76, 77]. The potential roles of the administered dose and morphological features of the VP6 structural assemblies on their adjuvant activity were also addressed in studies on immunization with norovirus VLP [77]. In this context, it was shown that despite higher induction of the cytokine IL-4 by co-administration of VP6 tubes with norovirus VLPs than with VP6 VLPs, both of these oligomeric VP6 assemblies exerted a similar dose-dependent adjuvant effect on norovirus-specific Ab responses [77]. More-recent studies have indicated that the particulate nature and size of the co-administered Ag might also affect the adjuvant effect of VP6 VLPs [55]. In this context, co-administration of VP6 VLPs with either a 20-nm norovirus P particle or a 23-mer extracellular domain of matrix protein M2 (M2e, a monomeric peptide, ~3 kDa) of human influenza A virus showed an adjuvant effect of VP6 VLPs only, enhancing Ab and T-cell immune responses against the preceding Ag (norovirus P particle), and not M2e [55]. VP6 tubes/VLPs have also been shown to enhance the uptake and presentation of co-administered norovirus GII.4 VLPs by dendritic cells [109, 110]. VP6 tube/VLP structures, and even their aggregates lacking high structural order, were efficiently internalized by bone-marrow-derived dendritic cells (BMDCs). Interestingly, compared to VP6 tube/VLP structures, an increased level of internalization was observed by the aggregates. Although the reasons behind these observations still remain to be explained, similar levels of IFN- γ production by splenocytes of VP6-immunized mice were detected regardless of the structural assembly [109]. Finally, it is worth mentioning that similar adjuvant effects were observed when plant-derived VP6 tubes were co-administered with plant-derived norovirus VLPs, indicating the independence of this adjuvant characteristic on the source of the cell/host used for vaccine production [78].

Overall, VP6 tubes/VLPs not only induce higher levels of immunogenicity compared to trimers or aggregates of VP6 but also have great potential to act as an adjuvant and to enhance prominent Ab and T-cell immune responses against foreign antigens.

VP6 tube/VLPs as immunological carriers

The strong immunogenicity and inherent immunostimulatory and immunomodulatory characteristics of VP6 tubes/VLPs make this structured protein assembly an important candidate vaccine-delivery platform for foreign/heterologous Ag presentation. The first reports on applications of RV VP6 spherical VLPs as antigen carriers were in the early 1990s, when heterologous peptides/proteins were efficiently coupled to these carriers and successfully used as strong immunogens to induce high titers of specific Abs against the coupled peptides/proteins without the use of adjuvants (Fig. 1B) [49, 102]. Since then, there have been many reports on the successful application of RV VP6 structured protein assemblies for chimeric Ag delivery, including insertion of (i) a 14-amino-acid peptide derived from the simian paramyxovirus 5 (V5 epitope) by genetic fusion [96], (ii) three epitopes of poliovirus type 1 (PV1) [92], (iii) three epitopes derived from the VP4 of RV [114], (iv) two peptides (23 and 140 amino acids) derived from the M2 and hemagglutinin (HA) genes of influenza A virus [53], and, more recently, (v) insertion of the receptor-binding domain (RBD) of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spike protein (S) to the surface loops or the N-terminus of VP6 without affecting protein conformation and immunogenicity [111]. The results of the immunization studies using these VP6-based chimeric Ag delivery systems in animal models have demonstrated the potency of VP6-based structured protein assemblies (VP6 tubes/VLPs) for induction of high titers of Abs and strong T-cell immune responses against both VP6 and the inserted Ag, suggesting the conformational conservation of both Ags in the chimera.

VP6 tubes/VLPs as drug-delivery tools

Besides serving as immunological carriers, RV VP6 structured protein assemblies in the form of nanotubes/VLPs are capable of encapsulating therapeutic reagents for the purpose of drug delivery (Fig. 1B) [122, 126]. Indeed, owing to their biocompatibility, biodegradability, and encapsulation characteristics, such viral nanoparticles are suggested to act as carriers of targeted therapeutics and anticancer vehicles [122]. In this context, it has been reported that self-assembled RV VP6 particles fused to the small ubiquitin modifier (SUMO) domain maintain their nanotube/VLP structure in

the gastrointestinal (GI) tract when administered orally and represent a suitable biodegradable carrier [5, 91, 122, 126]. These results suggest the potential application of self-assembled RV VP6 particles for drug delivery to the GI tract.

VP6 tubes/VLPs as nano-biomaterials

Biological molecules with the ability to self-assemble are highly ordered, symmetrical, homogeneous structures with various sizes and shapes [41]. Among the different biomolecules (nucleic acids, proteins, peptides, and viral particles), viral protein assemblies, which are highly ordered complex structures with multifunctional binding properties and devoid of genetic material, would be ideal as a new platform for the synthesis of natural nano-materials [41, 42]. One protein with these features is RV VP6, which is ideal for obtaining tubes/VLPs that can form multifunctional scaffolds for construction of organized nano-biomaterials (Fig. 1B) [23, 97, 99]. It has also been shown that RV VP6 in the context of trimer and nanotube assembly can act as a scaffold to allow charge transfer processes and to create bio-electrochemical interfaces [50].

The selection of VP6 assemblies as scaffolds for the synthesis of the metallic nanoparticles, functionalized *in situ* with metals such as silver (Ag), gold (Au), platinum (Pt), and palladium (Pd) was first reported in 2009 [99]. Although functionalization with metals usually results to the formation of nanotubes/particles with the metal attached to the external surface [97–99], reduction of the metal *in situ* to produce nanorods and nanowires inside the nanotubes has also been reported [23]. Finally, it is worth mentioning that molecular docking simulations have shown that exposed residues of RV VP6 are predicted to be able to bind to Pd (II) ions and to produce nucleation sites for the growth, stabilization, and control of Pd particles [24].

Overall, the accumulated data indicate that RV VP6 tubes/VLPs can be used as scaffolds to generate nanotubes with metal on either the external or internal surface. These properties might find important applications in nano-biotechnology.

Conclusions

The highly conserved and abundant structural protein VP6 of RV is an immunogen that is capable of self-assembling into nano-sized spherical and tubular forms when expressed in isolation under defined conditions. Different expression systems from prokaryotic *E. coli* to eukaryotic systems such as yeast, insect cell/baculovirus systems, mammalian cells, and plant cells have been used successfully to produce assembled VP6 structures such as VP6 tubes/VLPs. These

structures have been shown to be stronger immunogens than VP6 monomers for protecting vaccinated animals from RV infection and thus might be appropriate alternatives for RV vaccine formulations. VP6 tubes/VLPs also show adjuvant activity when co-administered with other Ags such as norovirus VLPs. This adjuvant activity, which involves enhanced uptake of the Ag into APCs, has been shown to be strictly dependent on co-localization of VP6 with norovirus VLPs. The applicability of VP6-tubes/VLPs as immunological carriers has been shown by fusing them to heterologous Ags from several viruses, such as polio virus, influenza virus, and SARS-CoV-2, and the successful delivery of the chimeric Ags. VP6 tubes/VLPs are biocompatible and biodegradable nanocompounds that are capable of encapsulating therapeutic reagents for treatment of diseases such as cancer. Finally, VP6 tubes/VLPs can form multifunctional scaffolds that can be used to create bio-electrochemical interfaces and for synthesis of organized nano-biomaterials and metallic nanoparticles. These properties of RV VP6 tubes/VLPs make them potentially suitable for various practical applications.

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Author contributions All authors contributed to the writing of the manuscript.

Declarations

Conflict of interest The authors declare no conflicting financial or other interests.

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