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Newcastle disease virus-like particles as a platform for the development of vaccines for human and agricultural pathogens

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Abstract

Vaccination is the single most effective way to control viral diseases. However, many currently used vaccines have safety concerns, efficacy issues or production problems. For other viral pathogens, classic approaches to vaccine development have, thus far, been unsuccessful. Virus-like particles (VLPs) are increasingly being considered as vaccine candidates because they offer significant advantages over many currently used vaccines or developing vaccine technologies. VLPs formed with structural proteins of Newcastle disease virus, an avian paramyxovirus, are a potential vaccine candidate for Newcastle disease in poultry. More importantly, these VLPs are a novel, uniquely versatile VLP platform for the rapid construction of effective vaccine candidates for many human pathogens, including genetically complex viruses and viruses for which no vaccines currently exist.

Keywords

human vaccines; Newcastle disease virus; vaccine platform; veterinary vaccines; virus-like particles

Challenges for vaccine development

Vaccination remains the most effective means of preventing virus infection in individuals and controlling spread of virus infections through a population. The generation of vaccines for many viral pathogens has had a major impact on global health. However, despite their significant benefits, many currently licensed vaccines have inherent problems that may result in reconsideration of their widespread use, particularly when the incidence of the targeted disease is significantly decreased. For other viral pathogens, classic approaches to vaccine development have, thus far, not been successful. For example, no licensed human vaccines are available for HIV, respiratory syncytial virus (RSV), arenaviruses, bunyaviruses, dengue virus, West Nile virus, cytomegalovirus (CMV), HCV or herpes simplex viruses. An additional challenge for vaccine development is the emergence of previously unidentified pathogens, such as the severe acute respiratory syndrome (SARS) or Nipah viruses, and variants of common pathogens, such as pandemic influenza viruses. Thus, there is a vital need for novel ways to develop safer vaccines for already targeted

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pathogens, vaccines for pathogens for which no vaccines exist, and rapidly produced vaccines for emerging threats. Virus-like particles (VLPs), and particularly versatile VLP vaccine platforms, such as Newcastle disease VLPs described in this article, are potentially a solution for development of alternative, safer vaccines or vaccines for previously intractable pathogens.

Current approaches to viral vaccines

Most licensed viral vaccines are either live, attenuated or inactivated viruses. Live, attenuated viruses are considered to be the most effective vaccines. However, live-virus vaccines may cause disease in immunocompromised individuals, a consideration of increasing concern, owing to the HIV pandemic and organ transplantation, as well as cancer chemotherapies. Live viruses also have the potential to revert to virulent forms, albeit at low frequency, or to recombine with endemic viruses to produce a potentially virulent virus [1,2]. Contamination of these vaccines with other viruses is still a problem, such as that which has been recently documented for one of the newly approved rotavirus vaccines [101].

Recombinant live avirulent viruses, containing foreign genes engineered into their genome, are also currently being developed as very promising vaccine candidates (reviewed in [3], for example [4,5]). However, live avirulent vector viruses may also pose risks in immunocompromised populations. In addition, vector viruses may stimulate undesired, abnormal immune responses (reviewed in [6]).

Inactivated vaccines are safer but produce poorer, shorter-lived immune responses than live virus, in part owing to alteration of the immunogenicity of the viral proteins during inactivation (reviewed in [7]). Furthermore, inactivated vaccine virus preparations require production of large quantities of virulent viruses, which, for certain viruses, require special containment measures and dangers to production personnel. Other viruses, such as the avian influenza virus, are difficult to produce in large quantities owing to their virulence [8,9] or their inefficient production in tissue culture systems. In addition, vaccination with some inactivated viruses, for example RSV and measles virus, actually exacerbated disease upon subsequent exposure to the live virus (reviewed in [7,10,11]).

Other types of vaccines in development are purified proteins, DNA, and replicon or replication-defective vaccines. Purified proteins are not usually effective without adjuvants, but few adjuvants are licensed for use in humans. Many DNA vaccines are under active development, but none have yet been licensed for use in humans (reviewed in [12]). Furthermore, in human trials, immune responses are reported to be weak without additional immunization [12,13]. Replicon vaccines are defective genomes into which a foreign gene is introduced (e.g., [14,15]). While capable of only a single cycle of infection, defective genomes may replicate or persist for extended periods in the infected cell, increasing the risk of recombination between the replicon and an endemic virus to generate a virulent virus [16].

VLPs as vaccines

Virus-like particles are increasingly being considered as viral vaccines (reviewed in [8,17,18]), although their potential has not been exploited. Only two VLP vaccines are currently licensed for use in human populations (described in [17]). VLPs composed of capsid proteins have been approved as a papilloma virus (HPV) vaccine. The HBV vaccine is a particle produced in yeast and is composed of a membrane and the HBV surface antigen. These vaccines do illustrate the two general types of VLPs: those formed with proteins from nonenveloped viruses and those formed with proteins derived from enveloped viruses.

Virus-like particle vaccines may offer significant advantages over many currently used vaccines or developing vaccine technologies [17]. VLPs are formed by the assembly of viral structural proteins and lipids into particles, but without the incorporation of the viral genome. Thus, VLPs are incapable of a spreading infection typical of an infectious virus, eliminating danger of disease in immunocompromised individuals. There is no chance of reversion to virulence or recombination with endemic viruses, since no genome is present. The production of VLPs is not hazardous, since no live virus is involved in their preparation. Importantly, VLPs are potent immunogens, since they are particles similar in size to viruses and contain repeating protein arrays on their surfaces and in their cores that mimic those of infectious viruses [17]. Such structures account, in part, to the potent immunogenicity of viruses [17]. Furthermore, since the assembly of particles, such as the assembly of viruses, requires correct folding of proteins, VLPs likely contain many authentic antigenic sites. Since no inactivation of VLPs is required, important epitopes are retained and new ones are probably not generated. Importantly, in contrast to many virus vaccines, VLPs may be formed without viral proteins that downregulate host immune responses [19].

Preclinical studies of VLPs in experimental animals

In addition to the licensed HPV and HBV VLP vaccines, a number of VLPs have been tested as potential vaccine candidates. Preclinical studies of immune responses and efficacy of many of these VLP vaccine candidates have provided very encouraging results. More recent examples of these studies include characterization of Ebola virus VLPs [20], seasonal and pandemic influenza VLPs [21–24], Chikungunya virus VLPs [25], HIV VLPs [18,26] and HCV VLPs [27]. As summarized in many excellent reviews (e.g., [8,17,18,26,28,29]), most well-characterized VLPs are potent stimulators of immune responses. Surface proteins usually stimulate neutralizing antibody. Owing to their particulate nature, VLPs are taken up and processed for presentation by both MHC class I and class II pathways, resulting in a broad range of T-cell responses. Of key importance, VLPs are described as ‘self-adjuvanting’ [28,29]. No adjuvant is required for immune responses in most systems. All these responses usually translate into vaccine candidates that provide impressive and safe protection of experimental animals from challenge by live, virulent viruses.

VLPs as vaccine platforms

Not all virus systems will produce VLPs. Other viral systems do not yield VLPs at levels likely to be sufficient for their use as immunogens. To extend the use of established, well-characterized VLPs as vaccines for other viruses, several approaches have been reported. The passive incorporation of glycoproteins into VLPs formed with retrovirus gag proteins has been reported (reviewed in [26] and illustrated in [30,31]). Immunodominant molecules can be chemically crosslinked to the surfaces of VLPs [17]. Finally, key neutralizing antibody or T-cell epitopes of a target virus have been genetically fused to a structural protein of a well-characterized VLP. There are many variations on this last approach (more recent examples include [17,26,28,32–34]), but most of these chimera VLPs have been reported for retrovirus gag, the HBcAg or the HBsAg VLPs. The efficacy of these chimera VLPs in protection from the targeted pathogen has been less extensively characterized.

Newcastle disease VLPs as poultry vaccines

We are developing VLPs formed with the structural proteins of the avian paramyxovirus Newcastle disease virus as vaccines and vaccine platforms. Paramyxoviruses are enveloped, negative-stranded RNA viruses [35–37]. All paramyxovirus virions (Figure 1) contain at least two glycoproteins: an attachment protein, termed hemagglutinin–neuraminidase (HN) protein, for Newcastle disease virus; and a fusion (F) protein, which directs the fusion of viral and cellular membranes. Lining the inner surface of the membrane is the matrix or

membrane (M) protein. The core of the virus contains the genomic RNA and three associated proteins: the nucleocapsid protein (NP), which binds to the RNA genome; a phosphoprotein (P); and the viral polymerase, the large (L) protein.

It has been reported that many different paramyxovirus VLPs can be produced upon expression of the M protein or M protein with various combinations of the glycoproteins and NP [32,38–43]. Indeed, as depicted in Figure 1B, cells expressing the Newcastle disease virus HN, F, NP and M proteins release particles that structurally and functionally resemble virus particles [44,45]. What distinguishes Newcastle disease VLPs from other paramyxovirus VLPs and, indeed, from many other types of VLPs, is their striking efficiency of release [45]. The reported efficiency of release of other paramyxovirus VLPs, as measured by the efficiency of M protein released, ranged from 10 to 50% [32,38–43], while Newcastle disease VLP release is 84% [44,45]. As a result, quantitative amounts of Newcastle disease VLPs are relatively easy to prepare even from transiently transfected cells [46]. They can be purified using protocols utilized for virus purification and the purified VLPs have minimal cell protein contamination (Figure 2) [44,46]. Furthermore, the ratios of viral proteins were similar to those in virus particles (Figure 2) [44].

Glycoproteins assembled into Newcastle disease VLPs are likely in their authentic conformation. The most stringent test of the conformation of a glycoprotein is the preservation of the biological activities of virion-associated glycoproteins. Indeed, HN protein associated with Newcastle disease VLPs mediates cell binding and possesses neuraminidase activity [44]. The HN protein associated with these particles also directs hemagglutination with titers comparable to equivalent amounts of virus [44]. F protein in these particles can direct the fusion of the VLP membrane with red blood cell membranes [44].

The effectiveness of Newcastle disease VLPs as an immunogen was demonstrated in a murine model and compared with responses stimulated by immunization with comparable amounts of a UV-inactivated, vaccine strain of Newcastle disease virus [44]. Levels of soluble antibodies, characterized by ELISA and by neutralizing antibody titers, resulting from Newcastle disease VLP immunization were as high or higher than those resulting from immunization with vaccine virus [44]. Furthermore, Newcastle disease VLPs stimulated T-cell responses at levels slightly higher than those stimulated by the vaccine virus [44]. Thus, Newcastle disease VLPs exhibit all of these characteristics of an effective vaccine and are potentially a veterinary vaccine candidate for Newcastle disease in poultry.

A current problem with available Newcastle disease virus vaccines is the failure of these vaccine viruses to protect against periodic outbreaks of virulent, exotic strains of the virus in domestic chicken flocks [47,48]. However, Newcastle disease VLPs have potential as a broad-spectrum Newcastle disease virus vaccine that could provide protection against many Newcastle disease virus strains, including these exotic strains, since we have found that the glycoproteins of different strains of Newcastle disease virus can be incorporated into these particles [44].

Newcastle disease VLPs as a vaccine platform

Newcastle disease VLPs have significant potential applications beyond Newcastle disease virus vaccines. Newcastle disease VLPs are a novel and uniquely versatile VLP platform for the rapid construction of effective vaccine candidates for many human pathogens, including genetically complex viruses. As described for other VLPs, Newcastle disease VLPs can be used to express peptide sequences from target pathogens. Indeed, we have shown that VLP assembly is unaffected by the genetic fusion of a foreign sequence to the amino terminus or the carboxyl terminus of the Newcastle disease virus NP protein or to the C-terminus of the

HN protein [44], raising the possibility of using these VLPs to present domains that can stimulate neutralizing antibodies or T-cell responses.

More importantly, Newcastle disease VLPs can be used to present entire ectodomains of glycoproteins from many different viruses. The Newcastle disease virus glycoproteins are assembled into VLPs owing to specific interactions of the glycoprotein cytoplasmic (CT) and transmembrane (TM) domains with Newcastle disease virus core proteins, while the ectodomain has little influence on assembly [Morrison TG *et al.*, Unpublished Data] [46]. Thus, specific incorporation of a foreign glycoprotein ectodomain into Newcastle disease VLPs can be achieved by constructing a chimera protein gene composed of sequences encoding the foreign protein ectodomain fused to those encoding the TM and CT domains of the appropriate Newcastle disease virus glycoprotein (Figure 3A). Both type 1 and type 2 glycoproteins can be assembled into these particles in this way, owing to the properties of the Newcastle disease virus glycoproteins. The F protein is a type 1 glycoprotein and the HN protein is a type 2 glycoprotein. Thus, foreign type 1 glycoprotein ectodomains can be fused to the TM and CT domains of the F protein while type 2 glycoprotein ectodomains can be fused to the CT and TM domains of the HN protein (Figure 3A). Specific assembly of the entire ectodomain of a protein rather than short peptides or domains more likely results in a properly folded protein with authentic post-translational modifications, conformational epitopes and T-cell epitopes, including those not yet identified.

Indeed, using this approach, we successfully incorporated into Newcastle disease VLPs the entire ectodomains of the Nipah virus G protein [44], the RSV G protein [46], both type 2 glycoproteins (Figure 3B), the RSV F protein, a type 1 glycoprotein, or both the RSV F and G proteins [Morrison TG *et al.*, Unpublished Data]. This approach is not limited to paramyxovirus glycoproteins. We have also incorporated into these VLPs the ectodomains of the influenza neuraminidase glycoprotein (type 2), the influenza hemagglutinin glycoprotein (HA; type 1), and the human CMV gB protein (type 1) [Morrison TG *et al.*, Unpublished Data]. Figure 3C illustrates the incorporation of the ectodomain of the CMV gB glycoprotein. Furthermore, we have incorporated into a single particle preparation the CMV gB and the RSV G protein ectodomains (Figure 3C), raising the possibility that Newcastle disease VLPs could be used as a single vaccine specific for two different pathogens. For example, assembly of the Newcastle disease virus HN protein and the influenza HA protein into a single VLP could be used to protect chickens from both avian influenza and Newcastle disease virus. Assembly of the RSV G protein and the influenza HA protein into a single particle could protect human populations from both RSV and influenza.

To test the potential of Newcastle disease VLPs containing foreign glycoprotein ectodomains as vaccines, VLPs containing the ectodomain of the RSV G protein or VLPs containing the ectodomains of both the RSV G and F proteins have been used as immunogens, without adjuvant, in a murine system. After immunization by either intraperitoneal or intramuscular inoculation, VLPs containing only the G protein ectodomain stimulated anti-RSV G protein antibodies at levels comparable or higher than levels resulting from live virus infection or immunization with UV-inactivated virus [46]. Immunization with these VLPs provided complete protection from RSV replication in lungs after intranasal challenge with live virus (Figure 4) [46]. VLPs containing the ectodomains of both the RSV G and F proteins stimulated, in mice, high titers of antibody to both the G and F proteins and high titers of neutralizing antibody responses [Morrison TG *et al.*, Unpublished Data]. These VLPs also provided complete protection from RSV replication after RSV challenge [Morrison TG *et al.*, Unpublished Data]. Owing to the fact that various forms of nonreplicating RSV immunogens, including formalin-inactivated virus (FI-RSV), have been reported to stimulate enhanced lung pathology upon live-virus challenge [11,49],

the effects of immunization with either of these VLP formulations on lung pathology was assessed. Neither VLP stimulated enhanced respiratory disease attributed to abnormal immune responses to FI-RSV in parallel immunizations (described in detail in Murawski *et al.* [46] [Morrison TG *et al.*, Unpublished Data], suggesting the safety of this type of RSV vaccine.

Additional considerations suggest that VLPs formed with Newcastle disease virus proteins would be effective yet safe human vaccines. Newcastle disease virus is an avian pathogen [47]. There are no reports that Newcastle disease virus causes human disease in the general population and there are no reports of seropositive human populations; thus, there is no previously existing immunity to interfere with immune responses. There is no evidence for adverse effects in humans due to the viral antigens. Indeed, live Newcastle disease virus has been injected intravenously in human clinical trials testing Newcastle disease virus as an oncolytic agent. These studies reported little to no toxicity [50]. Newcastle disease virus VLPs would, therefore, be an ideal framework on which to base human vaccines.

Future perspective

It is likely that, in the near future, many more VLPs will be licensed for use as vaccines in both humans and agricultural animals. Newcastle disease VLPs, as well as other types of VLPs, show significant promise in preclinical studies. The next step in the translation of these positive preclinical results into vaccine trials and into licensure as human and agricultural vaccines requires that VLPs be produced in a cost-effective manner, on a large scale, and under good manufacturing processes. This step is the next major challenge for continued development of VLPs as vaccines, particularly enveloped VLPs (reviewed in [51]), such as Newcastle disease VLPs. However, VLPs formed with influenza proteins and with Ebola virus proteins can be produced from insect cells using baculovirus vectors (e.g., [20,22,24]), a system that should be easily adaptable to large-scale production for many VLPs. Issues surrounding VLP production from baculovirus vector-infected insect cells involve the altered carbohydrate modification of glycoproteins synthesized in insect cells (discussed in [52]) and contaminating baculoviruses in the VLP preparations (discussed in [28,53]). However, influenza VLPs formed in this way are in clinical trials (NCT01072799, NCT01014806, NCT00903552 and NCT00519389) [102,103]. Alternatively, systems utilizing tissue culture cells approved for use in vaccine production could also be adapted for production of VLPs. Cell lines derived from these approved cells expressing VLP proteins may be produced. Alternatively, large-scale transient transfections are being explored. Purification of VLPs from mammalian cells would eliminate any issues with immunogenicity associated with the altered post-translational modifications of proteins produced in insect cells and would eliminate concerns about the presence of baculoviruses in the vaccine preparations.

It has been noted that some vaccine candidates can stimulate very effective immune responses in animal models but fail to do so in humans, a problem exemplified by some clinical trials of potential human RSV vaccine candidates (reviewed in [54]). However, many of the VLP vaccine candidates that have been tested in human trials do stimulate robust immune responses, indicating that this approach to vaccine development has great potential. Furthermore, characterization of immune responses to VLPs, including chimera Newcastle disease VLPs, in newly developed humanized mouse models may facilitate the path of VLP vaccine candidates to clinical trials. These systems have the potential to assess more directly human immune responses [55–57] during preclinical development of VLP vaccine candidates.

Executive summary

Challenges for vaccine development

- New safer vaccines are needed to replace old ones.
- Vaccines need to be developed for pathogens for which no vaccines exist.
- Ways to rapidly produce vaccines for newly emerged threats need development.

Current approaches to viral vaccines

- Live, attenuated virus vaccines are effective, but have dangers.
- Inactivated virus vaccines are usually safer, but are often less effective.
- Many of the newer approaches to vaccine development also have inherent problems.

Virus-like particles as vaccines

- Virus-like particles (VLPs) are particles that resemble virus particles, but they have no genome.
- VLPs are very immunogenic.
- VLPs as vaccines offer many advantages over other vaccine technologies.

Preclinical studies of VLPs in experimental animals

- VLPs stimulate neutralizing antibodies and diverse T-cell responses.
- No adjuvants are required for immune responses stimulated by VLPs.

VLPs as vaccine platforms

- Some VLPs can be used as a platform to present antigens from different pathogens.

Newcastle disease VLPs as a vaccine for Newcastle disease

- VLPs are released with striking efficiency from cells expressing the Newcastle disease virus nucleocapsid, membrane, hemagglutinin–neuraminidase and fusion proteins.
- Newcastle disease VLPs resemble the virus and the glycoproteins retain their activities.
- Newcastle disease VLPs stimulate humoral and cellular immune responses comparable to the vaccine virus.
- Glycoproteins from different strains of Newcastle disease virus can be incorporated into Newcastle disease VLPs.

Newcastle disease VLPs as a vaccine platform for human vaccines

- Newcastle disease VLPs are a uniquely versatile vaccine platform.
- Short foreign sequences can be genetically fused to the ends of the nucleocapsid or hemagglutinin–neuraminidase protein.
- The entire ectodomain of different foreign glycoproteins can be assembled into Newcastle disease VLPs.

- Chimera Newcastle disease VLPs stimulate immune responses comparable to live virus infection.
- Immunization with chimera Newcastle disease VLPs provides complete protection upon live virus challenge in an animal model.

Future perspective

- Increasing numbers of VLP vaccines will likely be licensed in the near future.
- Development of Newcastle disease VLPs as vaccines requires their large-scale production.
- The effectiveness of Newcastle disease VLPs as vaccines in humans will be explored utilizing humanized mouse models.

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Figure 1. Paramyxovirus virions and the generation of Newcastle disease virus-like particles
(A) Paramyxovirus virions. *Paramyxoviridae* are enveloped, negative-stranded RNA viruses. The viral membrane (lipid) contains two glycoproteins, an attachment protein and a F protein, and a nonglycosylated M protein. The attachment protein of Newcastle disease virus, an avian paramyxovirus, is the HN protein. The core of the virus contains the genomic RNA and associated nucleocapsid protein in a helical RNP complex, which also contains the L viral polymerase and a P protein. **(B)** Generation of ND VLPs, which are very efficiently released from cells expressing the viral NP, M, HN and F proteins after a transient transfection of cells with pCAGGS vectors [58,59] containing cDNAs encoding each of these viral proteins. These cDNAs were derived from the Australia–Victoria strain of Newcastle disease virus. The cleavage site of the F protein was mutated to mimic the site in the F protein from the vaccine strain of Newcastle disease virus, resulting in an uncleaved F protein. Cells may be mammalian (COS7 or 293T) or avian cells (ELL-0), although avian cells release Newcastle disease VLPs more efficiently. In avian cells, 84% of total expressed M protein is released as particles [45].
 F: Fusion; HN: Hemagglutinin–neuraminidase; L: Large; M: Membrane; ND: Newcastle disease; NP: Nucleocapsid protein; P: Phosphoprotein; VLP: Virus-like particle.

Figure 2. Protein content of purified Newcastle disease virus-like particles compared with virus VLPs harvested from the supernatant of cells expressing the Newcastle disease NP, M, HN and F proteins are purified by sequential sedimentation and flotation in protocols similar to those used for virus purification, as described in Pantua *et al.* [45] and McGinnes *et al.* [44]. Proteins in the purified VLPs, separated by electrophoresis on polyacrylamide gels and detected by silver stain, are compared with proteins in equivalent amounts of purified, avirulent vaccine virus grown in embryonated chicken eggs. Proteins shown in lanes 1 and 2 were separated on polyacrylamide gels in the absence of reducing agent ($-\beta$ ME) in order to resolve the disulfide linked F_1-F_2 heterodimer of the cleaved form of F protein present in egg-grown virus and the disulfide-linked HN homodimer of the correctly folded HN protein of the AV strain of Newcastle disease virus. The HN protein of the vaccine strain is not disulfide linked.

β ME: β -mercaptoethanol; F: Fusion; F_1 : Cleaved form of the F protein; Fnr: Nonreduced form of F protein (F_1+F_2); HN: Hemagglutinin-neuraminidase; HN dimer: Disulfide-linked HN protein; M: Membrane protein; NP: Nucleocapsid protein; VLP: Virus-like particle. Adapted with permission from [44] © American Society for Microbiology.

Figure 3. Incorporation of ectodomains of foreign glycoproteins into Newcastle disease virus-like particles

(A) Illustrates the rationale for the construction of chimera glycoprotein genes. For type 1 glycoproteins, the ectodomain of the NDV F protein is substituted with the ectodomain of a foreign glycoprotein X. For type 2 glycoproteins, the ectodomain of the NDV HN protein is substituted with that of a foreign glycoprotein Y. (B) Demonstrates the assembly into Newcastle disease VLPs of a type 2 chimera glycoprotein, a chimera protein containing the ectodomain of the RSV G protein and the CT and TM domains of the HN protein (H/G). Cells were transfected with cDNAs encoding the NDV NP and M protein, as well as cDNA encoding the chimera protein H/G. While M protein is both necessary and sufficient for Newcastle disease VLP release, inclusion of NP significantly enhances incorporation of glycoproteins into particles, particularly when only a single glycoprotein is expressed, as described in Pantua *et al.* [45]. Proteins in purified VLPs, separated by electrophoresis on polyacrylamide gels and detected by silver stain (lane 2), were compared with proteins in purified virions (equivalent amounts of protein were loaded onto lanes 1 and 2; NDV, lane 1). Lane 3 shows material released from equivalent numbers of cells transfected with an empty vector. Lanes 4 and 5 show a WB of VLP-H/G (lane 5) and virus (lane 4) proteins using anti-RSV antibody to detect the RSV G protein sequences in the VLPs. The H/G protein, as well as the RSV G protein (not shown), migrates with heterogeneous sizes most likely due to inefficient glycosylation [46]. (C) Illustrates the incorporation of a type 1 chimera glycoprotein into Newcastle disease VLPs. A chimera protein containing the ectodomain of the cytomegalovirus gB protein and the TM and CT domains of the NDV F protein (gB/F) was coexpressed with the NDV NP and M proteins (lane 3) or coexpressed with NDV NP, M, and H/G chimera protein (lane 4). VLPs containing the NDV HN, F, NP and M proteins are shown in lane 2, while particles released from empty vector DNAs are shown in lane 1. Panels show WBs of the proteins in purified VLPs. All lanes contained an amount of VLPs released, in the same experiment, from equivalent numbers of transfected cells. Efficiency of release of the different VLPs was similar to that demonstrated by similar levels of NDV NP in each preparation. Anti-CMV antibodies detect the gB sequences. Anti-NDV F CT is an antibody raised against the CT domain of the NDV F protein and demonstrates the presence of the NDV F protein CT domain sequences in the VLPs. Anti-NDV detects the NP protein in the VLPs and anti-RSV detects the RSV G protein sequences in the VLPs.

CMV: Cytomegalovirus; CT: Cytoplasmic; F: Fusion; HN: Hemagglutinin-neuraminidase; M: Membrane; NDV: Newcastle disease virus; NP: Nucleocapsid protein; RSV: Respiratory syncytial virus; TM: Transmembrane; VLP: Virus-like particle; WB: Western blot.

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Figure 4. Protection of experimental animals from live virus challenge after immunization with chimera virus-like particles

VLPs containing the H/G chimera protein and the Newcastle disease virus nucleocapsid protein and membrane protein were used as an immunogen in BALB/c mice as previously described [46]. VLPs (30 µg total VLP protein/animal) were delivered by intraperitoneal inoculation. Similar results were obtained using intramuscular immunization [46]. In parallel, groups of mice received no immunization, or were infected with live RSV (3×10^6 PFU/animal) delivered by intranasal immunization. Mice were challenged with infectious RSV (3×10^6 PFU/animal) and virus titers in lungs were measured at 4 days postchallenge. Results show virus titers/g of lung tissue for each mouse in each group.

RSV: Respiratory syncytial virus; VLP: Virus-like particle.

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