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Review

Virus-like particle and synthetic nanoparticle-based vaccines for paramyxoviruses

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ABSTRACT

Many human pathogens such as the highly infectious measles virus (MV), the clinically important respiratory pathogens like human respiratory syncytial virus (RSV), human metapneumovirus (hMPV), human parainfluenza virus 3 (hPIV3), the highly lethal zoonotic viruses, Nipah (NiV), and Hendra (HeV) are all paramyxoviruses in the family *Paramyxoviridae*. Despite their clinical importance, human-use vaccines are available for only two of these viruses, namely for MV and mumps; both were empirically derived many years ago. For the many others such as RSV, hMPV and hPIV3 vaccines have remained elusive in spite of concentrated efforts of many investigators for a long time, for decades in some cases. Vaccine, or at the very least vaccine preparedness is needed also for some other paramyxoviurses like NiV. Traditionally many viral vaccines have been based on inactivation or attenuation. While in general they are highly effective, in some cases they fail to provide adequate immunogenicity and safety, and may even cause adverse events. In the case of live attenuated vaccines, achieving a stable optimally attenuated virus is often difficult and there is the potential for reversion. Transmission to the immunocompromised individuals is an additional concern. Inactivated vaccines run the risk of producing enhanced disease. Subunit vaccines are poorly immunogenic and would require carefully selected adjuvant(s) to achieve protection. In this article, while we touch on some of these traditional vaccine approaches, our primary focus is to review the more recent potentially safer and more effective particulate vaccines such as virus-like particles, virosomes and synthetic nanoparticles for paramyxovirses.

KEYWORDS: paramyxoviruses, vaccines, VLPs, nanoparticles

INTRODUCTION

Paramyxoviruses belong to the family *Paramyxoviridae*. This large family of viruses includes some of the most clinically relevant human pathogens such as measles, mumps, RSV, hMPV, hPIV3, NiV, and livestock pathogens such as CDV, NDV, RPV and HeV. Of these, RSV and hMPV are in the subfamily *Pneumovirinae*, the rest are in the subfamily *Paramyxovirinae* (Figure 1).

Protective immune response through vaccination is the most effective strategy to prevent infectious diseases and this is borne out by the fact that vaccination is the single most cost-effective medical intervention ever. Human diseases such as smallpox, measles and polio which terrorized humankind have been controlled by vaccination. Today licensed vaccines are available for some 30 microbes, and millions of lives have been saved as a result. However, infectious diseases still take a heavy toll on human lives and millions die from vaccine preventable diseases. Many others die because there are no vaccines for several pathogens, and this in spite of concentrated efforts of many for a long time [1, 2, 3].

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Family: *Paramyxoviridae*

Subfamily: Paramyxovirinae - 5 genera:
- **Respirovirus:** Sendai virus (SeV); human parainfluenza 1 and 3 (hPIV 1 and 3)
- **Rubulavirus:** Mumps virus; hPIV2; hPIV4
- **Avulavirus:** Newcastle disease virus (NDV)
- **Morbillivirus:** Measles virus (MV); canine distemper virus (CDV); Rinderpest virus (RPV)
- **Henipavirus:** Hendra virus (HeV); Nipah virus (NiV)

Subfamily: Pneumovirinae - 2 genera:
- **Pneumovirus:** human respiratory syncytial virus (RSV); bovine RSV (bRSV)
- **Metapneumovirus:** human metaneumovirus (hMPV)

Menangle, Tioman (Rubulavirus), J virus, Beilong (new genus)

Figure 1. Members of the *Paramyxoviridae* family. *Praramyxoviridae* includes the two subfamilies *Paramyxovirinae* and *Pneumovirinae*, and the various genera and species as shown.

More recently, reverse vaccinology has resulted in expedited identification and evaluation of protective antigens. Also, research into molecular biology, immunology and vaccinology has provided new understanding on mechanisms of virus-host interactions that is proving to be essential for developing newer, safer vaccines. Purified viral proteins, genetic engineering and improved information about correlates of protection permit the creation of rationally designed attenuated mutants, and live and replication defective viral vectors expressing genes of interest. It is now also possible to manipulate viral DNA, proteins, and even polysaccharides and RNA to stimulate an array of immune responses. Particulate vaccines that incorporate viral properties are likely to induce tailor-made responses, and therefore have the potential to provide safer and more effective preventive as well as therapeutic vaccines [1, 2, 3, 4].

Vaccine strategies

Some of the vaccine strategies currently used include inactivated vaccines, live attenuated vaccines, replication competent and replication defective viral vector vaccines, DNA vaccines, and subunit vaccines. Many human-use viral vaccines licensed decades ago are either empirically-derived live attenuated vaccines such as measles and mumps, or inactivated ones such as rabies vaccine, and Salk vaccine for polio. The newer particulate vaccines such as virus-like particles (VLPs), virosomes, and synthetic nanoparticle vaccines are also being evaluated, and two native VLP-based vaccines, namely for Hepatitis B virus (HBV) and human papillomavirus (HPV) have already been licensed.

A. Conventional vaccine strategies

Inactivated viral vaccines

Inactivated viral vaccines have been used effectively in some cases like the formalin inactivated Salk vaccine for polio. However, not all inactivated virus vaccines are safe and effective, and some may even cause exaggerated disease. A case in point is the formalin inactivated alum-adjuvanted RSV vaccine clinical trial undertaken in the late 1960s. This was the first attempt at making an RSV vaccine, and unexpectedly, the children vaccinated with this preparation (FI-RSV) developed enhanced disease rather than protection when subsequently exposed to the wild type virus; eighty percent of them had to be hospitalized, and two died [5]. It has recently been reported that the enhanced disease occurred because of lack of antibody affinity maturation due to poor Toll-like receptor (TLR) activation and suboptimal recognition of the relevant protective RSV epitopes [6]. Moreover, the Th2-biased memory recall response caused increased infiltration of eosinophils in the lung, and consequent lung injury [7]. Formalin inactivated vaccines for other paramyxoviruses,

namely hMPV and hPIV3 also showed enhanced disease upon subsequent exposure to the wild type virus [8, 9, 10]. Since then, for all these reasons, the inactivated vaccine approach has been abandoned for all paramyxoviruses.

Live attenuated vaccines

Live attenuated vaccines have proved to be highly effective immunogens inducing long-lasting T and B cell immunity. Such vaccines are acceptable for the vast majority of the population but there is the risk of transmission to the non-targeted individuals such as the immunocompromised with the associated safety concerns. Most importantly, there is the potential risk of reversion to virulent virus. Nevertheless, the empirically derived live attenuated viral vaccines such as for measles and mumps viruses are clearly stably attenuated as they have been used successfully for decades [11].

Strategies based on rational design of live attenuated vaccines such as mutating the transcription/ replication control elements of the virus, inserting attenuating point mutations corresponding to the host range, deleting the "non-essential genes" of the virus, and others based on genetic modifications have been evaluated [12, 13, 14, 15, 16, 17]. Another approach has been the deletion of non-structural interferon antagonist genes which serve as virulence factors. This results in attenuated viruses that are now less able to counter host immune responses [18, 19, 20, 21]. Attenuation is also achieved when promoter regions of closely related viruses are switched; we demonstrated this by evaluating minigenome chimeras of MV and CDV (our unpublished data). The validity of this approach was confirmed later by Chapman *et al.* [22]. However, none of these approaches have come to fruition so far. During preclinical and clinical testing some viruses were found to be either over attenuated, or under attenuated, or the attenuating mutations were not stable and had the potential for reversion. In this regard, the effect of attenuating point mutations should be viewed with caution since it is possible that compensatory changes may have been introduced elsewhere to negate the effects of the designed mutations [23, 24]. Regardless, a stable live attenuated RSV vaccine (ΔNS2/Δ1313/1314*L*) is being tested in Phase 1 clinical trial [24]. A Phase 1 trial is ongoing also for RSV M2-2 deletion mutant (Medi-RSVΔM2-2) delivered intranasally in adults and seropositive and seronegative children [25, 26].

Recently, newer approaches such as microRNA-controlled, and zinc finger nuclease-controlled live attenuated vaccines for RNA and DNA viruses, respectively have been described and have received a lot of attention. However, they require further testing *in vitro* and in animal models before progressing further [11].

Replication competent vectors

Efforts to generate vectored vaccines continue, and several non-segmented negative-stranded RNA as well as DNA viruses have been used to express vaccine antigens; these include vaccinia virus [27], adenovirus [28], SeV [29], hPIV3 [30], MeV [31], NDV [32, 33], vesicular stomatitis virus [34, 35, 36] and others. However, there are concerns with some about preexisting antibodies to the vector. Also, similarly to the live attenuated vaccines, an additional concern is the potential of some of these vectors to cause disease in persons with impaired immunity.

Replication defective vectors

Replication defective vectors such as alphavirus and adenovirus vectors carrying RSV genes have been reported [37, 38]. Alphaviruses are plus sense single-stranded RNA viruses that include Venezuelan equine encephalitis virus (VEE), Sindbis virus and Semliki forest virus, and they have been used effectively to express genes of interest. While the nonstructural genes in these viruses are translated from the 5' end of the genomic RNA, the structural genes are encoded from the subgenomic promoter present only in the negative-stranded replication intermediate. Deletion of the viral structural genes and replacing them with the gene(s) of interest results in a propagation-defective virus that cannot spread to the adjoining cells, but results in a self-replicating RNA (replicon) expressing large amounts of heterologous proteins. The replicon system is attractive for vaccine development for several reasons, among them safety and high expression of vaccine antigen [39]. Systemic immunization of VEE replicon vaccine expressing RSV surface glycoproteins is known to induce protective mucosal responses in

mice and cotton rats [40]. As for adenovirus vectored vaccines, this subject has been discussed in some depth by Graham [41].

Subunit vaccines

Subunit vaccines composed of recombinantly-expressed purified proteins are safer, but the tradeoff is that they are in general less immunogenic than live attenuated or inactivated vaccines. They are not recognized by PRRs (pathogen recognition receptors) and so are not able to activate effective innate immunity and consequently are unable to induce appropriate adaptive immunity. Also, soluble subunit protein antigens are less effective than particulate immunogens and can suffer from suboptimal presentation to the immune system; they are not able to be cross-presented to MHC I molecules, and therefore are unable to induce cytotoxic T cell immunity [3]. Nevertheless, RSV F subunit vaccine continues to be of interest since it has been shown recently that mice vaccinated with stabilized pre-fusion RSV F protein at 10 µg, produced neutralizing antibody levels many times the protective threshold [42]. For subunit vaccines, an adjunvant would usually be required to induce an effective adaptive immune response and in the study mentioned above, the pre-fusion RSV F protein was formulated with 50 µg of polyinosinic-polycytidylic acid stabilized with polylysinecarboxy-methylcellulose (poly-ICLC) adjuvant. To date, no soluble subunit viral vaccine has been licensed for human use [41] although several have been evaluated [43, 44, 45, 46, 47].

B. Particulate vaccine strategies

Recently the move has been towards generation of potentially safe and effective particulate vaccines with virus-like properties such as VLPs, virosomes, and synthetic nanoparticles.

VLPs

Expression of one or more viral structural proteins from cDNA results in spontaneous assembly of particles that resemble the real virus morphologically and immunologically. These particles are safe because they are devoid of any viral genetic material, and therefore not infectious. VLP vaccine strategies show considerable promise because they could potentially produce both humoral and cell-mediated immunity and protection [3, 48]. Unlike soluble proteins, particulate antigens like VLPs, with their virus-like dimensions are taken up by phagocytosis and are cross-presented efficiently to reach MHC 1 pathway resulting in priming of CD8+ T cells. VLPs in particular are also highly effective in generating a protective antibody response because of their size range and particulate nature and because of their repetitive and ordered surface structure. The spacing of the antigenic epitopes on the VLPs also appears optimal for B cell activation [3, 48, 49]. For all these reasons, VLPs would be expected to be highly effective as prophylactic vaccines for paramyxoviruses where neutralizing antibody response plays a pivotal role in protection against disease [50, 51, 52].

Two major classes of VLPs have been described for vaccine development. They are a) native VLPs where one or more of the constituent viral proteins serve as immunogens, and b) carrier VLPs which are derived from a variety of unrelated sources such as plant or animal viruses, and function as carriers of foreign antigens/epitopes of interest [48, 53]. Native VLPs that include the surface glycoproteins are conformationally authentic and allow the vaccine essential proteins to be presented in their unaltered form. They are widely explored as prophylactic antiviral vaccines, and their potential as vaccine is underscored by the fact that the VLP-based vaccines for HPV, namely Gardasil (Merck & Co) and Cervarix (GlaxoSmithKline), have already been licensed and have proved to be extremely effective. HBV vaccine Recombivax-HB (Merck & Co) licensed in 1986 is also a VLP-based vaccine incorporating its surface antigen (HBsAg). However unlike the highly ordered surface structure of the classical VLPs, these VLPs have a complex irregular lipoprotein structure resembling the highly immunogenic "Dane" particles present in the sera of HBV infected persons [54]. The many other VLPs for a diverse group of viruses are at various stages of development [55, 56, 57].

Another class of VLPs has also been described and is based on coat protein of bacteriophage Qβ. This VLP approach is being used mainly for developing therapeutic vaccines for chronic diseases [53, 58].

Native VLP-based vaccines

Native VLP-based vaccines have been evaluated for three paramyxoviruses, namely RSV, NiV and

NDV. RSV infection is of public health concern worldwide. It is the single most common cause of bronchiolitis and pneumonia in childhood. Globally, in children under five years, the estimated RSV disease burden is over 30 million lower respiratory tract infections, some 3 million hospitalizations, and 160,000 deaths every year [59]. It is also an important cause of morbidity and mortality in the elderly and at risk adults [60, 61]. A vaccine has remained unattainable in spite of concentrated efforts of many investigators over some four to five decades. The development of vaccine for this virus has been challenging for several reasons [41]. Particularly challenging has been the failed formalin activated vaccine (FI-RSV) evaluated in clinical trials in the late 1960s. Vaccinated infants who were subsequently exposed to the wild type virus ended up getting enhanced disease rather than protection, many had to be hospitalized and two died [5]. This impaired vaccine has had a negative impact on later vaccine development: Safety concerns persist to date.

Experimental evaluation of native RSV G or RSV F VLPs produced by a baculovirus expression system was reported by a group at Emory University [62]. Two lots of VLPs were produced, one expressing RSV G, and one expressing RSV F protein. The resulting RSV G or RSV F protein VLPs both had influenza virus matrix protein M as the core. Mice were vaccinated with each of these two VLP vaccines intramuscularly twice at 4-week intervals with 25 µg VLPs/dose, challenged one week after the 2nd dose, and euthanized 4 days later. Neutralizing antibody response measured by plaque reduction neutralization titer ($PRNT_{50}$) was > 3(Log10), for both vaccines and both provided protection in the lungs. Of note, neutralizing antibody response at > 3(Log10) was higher than normally seen with non-replicating particles. Residual baculovirus present in the purified VLP preparations is known to activate innate immune response at the site of vaccination and enhance immunogenicity [63], and is likely to have been the reason for this augmentation. Also, in this study, RSV G vaccine showed better protection than the RSV F vaccine based on virus load in the lungs and morbidity in the animals post-challenge. One explanation given for this unexpected result was the likely differential glycosylation of the G and/or F proteins in insect cells relative to the mammalian cells.

Native VLPs composed of RSV A2 M, F and G proteins, and produced by transient transfection in mammalian cells have also been evaluated. Cotton rats (CRs) were vaccinated with two doses of these purified particles, 25 µg/dose adjuvanted with alum and monophosphoryl lipid A (MPLA). The animals were challenged with live virus three week after the second dose and euthanized 4 days post-challenge. The data (to be published) shows robust neutralizing antibody production and protection from replication in the lung as well as the nose of the vaccinated animals. VLP vaccine-induced virus clearance in the upper respiratory tract has not been reported previously with any VLP, virosomal or nanoparticle vaccines, and is an important consideration if the target population is < 6 months. This is because continued virus replication in the nose results in nasal congestion and babies at this age are essential nose breathers [26].

NiV is a zoonotic paramyxovirs and the causative agent of highly lethal febrile encephalitis in humans, predominantly respiratory disease in pigs (intermediate host), and no disease in fruit bats (reservoir host). The continued activity of this virus in Bangladesh, its highly lethal nature (case fatality rate between 60-90%), person-to-person transmission in one-half of recognized NiV case patients, and its potential as an agent of agroterror are all concerns heightened by the fact that there is no effective way to either treat or prevent disease caused by this virus [64, 65, 66, 67, 68]. Immunogenicity and vaccine potential of native NiV VLPs composed of two surface glycoproteins G and F, and the matrix protein M, all retaining their native properties has also been reported previously. *In vitro* study of these particles demonstrated their virus-like surface structure and their functional assembly and immunoreactivity (Figure 2). Mice vaccinated with as little as 7 µg of NIV VLPs, without the benefit of an adjuvant, were consistently able to induce neutralizing antibody response in every animal [49]. Evaluation of its protective efficacy in the hamster model of NiV disease is to be undertaken shortly.

NDV, an avian paramyxovirus, causes disease in many species of birds including domestic poultry

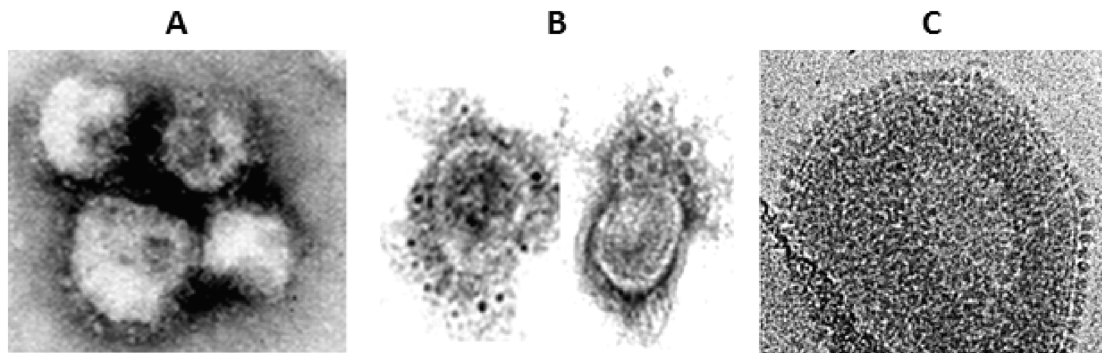

Figure 2. Nipah virus VLPs viewed by transmission electron microscope and CryoEM. Negatively stained sample in **(A)** shows well preserved VLPs; the spikes of the surface glycoproteins are clearly visible on the VLP surface. **(B)** Unfixed particles were stained by immunogold labeling technique using NiV-specific polyclonal antibody and gold-labeled secondary antibody. Unfixed particles were used so that only the surface glycoproteins would be available for immunoreactivity. The figure shows two VLPs with gold-decorated glycoproteins on the VLP surface. **(C)** Shows cryoelectron micrograph of one of the VLPs. The glycoprotein spikes and their spatial arrangement are seen here even more clearly (Adapted from Walpita, P., Barr, J., Sherman, M., Basler, C. F. and Wang, L. 2011, PLoS One, 6, e18437).

and it is highly contagious. It can also be transmitted to humans. Contact with infected birds may cause mild conjunctivitis and flu-like symptoms but otherwise it is not hazardous to humans. Immunogenicity has also been reported for VLPs of this virus [69]. In this study, native NDV VLPs composed of NP, M, HN and F proteins were produced in avian cells. Mice were immunized and boosted by intraperitoneal inoculation with 10, 20 and 40 µg of these VLPs. Neutralizing antibody response in pooled serum samples was reported.

Chimeric/carrier VLPs

In a study by McGinnes *et al.*, NDV VLPs containing the RSV F and G protein ectodomains fused respectively to NDV F and HN protein transmembrane, and cytoplasmic domains were created by reverse genetics. Balb/c mice immunized with 10 µg and 40 µg of these VLPs delivered intramuscularly induced neutralizing antibody titer of between 5 and 6(Log2). Intranasal challenge with live virus provided protection from replication in the lung and there was no enhanced disease as evidenced by Th1-biased immune response and inflammation scores in the lung [70]. In a previous study, the same group generated chimeric VLPs incorporating RSV G protein fused to NDV transmembrane and cytoplasmic domain of HN protein. Immunization of mice with either one or two doses of 10 µg or 40 µg of these VLPs in an ELISA-based assay induced soluble antibody titers of ~1/1,000 while neutralizing antibody titer was ~1/10. These antibodies also provided protection from replication in the lung, with no evidence of enhanced disease in the lung [71].

Particulate RSV F protein vaccine

This particulate vaccine was generated by recombinantly expressed RSV F protein in Sf9 cells. It was designed to produce cleaved and covalently linked F1 and F2 peptide homotrimers. When extracted from the host cell membrane and purified, the homotrimers self-assembled into rosette-shaped 40 nm particles composed of F protein oligomers. Cotton rats vaccinated intramuscularly with two doses of 30 µg of this preparation adjuvanted with alum induced high titered neutralizing antibody response [$PRNT_{100}$ = ~7-8.5(log2)], and prevented virus replication in the lung of the animals. Histopathology of lungs showed no signs of disease enhancement [72].

Virosomal vaccines

Virosomes are viral envelopes minus their nucelocapsids. They are produced by dissolving viral envelopes in detergent or short-chain phospholipid. The viral nucleocapsids and non-membrane proteins are removed by ultracentrifugation, and the viral membrane is reconstituted when the

detergent or short-chain phospholipid is removed. The resulting structure containing the viral membrane, membrane-integral proteins and lipids are called virosomes. Lipids, heterologous antigens/ proteins, adjuvants, or other materials, can be included during reconstitution of the virosome. The membrane-integral viral proteins confer structural stability to virosome-based vaccines [73, 74]. Almost all virosomal vaccines are composed of influenza virus or RSV envelopes. Since virosomes enter the cells similarly to the parental virus, they are able to deliver the cargo directly into the cell cytoplasm.

RSV envelope-based virosomal RSV vaccines

In vitro as well as *in vivo* analysis showed that RSV-based virosomal vaccine incorporating MPLA produced increased immunogenicity and protection relative to the non-adjuvanted RSV virosomes. The adjvanted vaccine signaling through TLR-4 was strongly stimulated relative to the RSV virosomes alone. Similarly, upregulation of co-stimulatory molecules CD40, CD80 and CD86 on dendritic cells was greater in the adjuvanted formulation. Mice vaccinated with RSV virosomes containing this adjuvant resulted in increased virus neutralizing titers and produced Th1-biased response as evidenced by the induction of IgG2a predominant response. In addition, it induced significant amounts of INF-γ and low amounts of IL5 in spleen as well as lungs. It also protected the lungs from the challenge virus. The same group did an additional study in cotton rats to show that MPLA adjuvanted virosomes induced responses similar to those in mice: The cytokine response in the lungs was Th1-biased, and provided protection with no evidence of disease enhancement in the lower respiratory tract [75, 76]. A subsequent publication showed that intranasal immunization of RSV virosomes adjuvanted with TLR-2 and NOD/2 ligands augmented serum antibody response, boosted mucosal immune response, and protected mice from developing disease. There were no signs or symptoms of enhanced disease or lung pathology [77].

Influenza envelope-based virosomal RSV vaccines

Influenza virosomal vaccine incorporating RSV F protein and adjuvanted with *Escherichia coli* heat-labile toxin was administered intranasally in the mouse model. This vaccine induced IgG, IgA, a robust neutralizing antibody response (mean $\pm$ SD of 6 mice was 6.9(Log2) $\pm$ 1.1), and a balanced Th1/Th2 cytokine response. This vaccine also induced cytotoxic T cell response. Histopathology of the lung demonstrated no evidence of pulmonary eosinophilia [78]. Another influenza virosome vaccine incorporating 10 µg or 50 µg of RSV F subunit protein was reported by Openshaw *et al.* at the 7th International RSV Symposium in 2010 (Symposium Proceeding). Two or three doses of the vaccine were delivered in mice subcutaneously at three week intervals. Mice developed neutralizing antibody response and protection from replication of the challenge virus. A follow up study was planned since in this study, surprisingly, even empty virosomes provided some protection. Biotechnology companies with virosomal vaccine platform include Mymetrics, Crusell, and Pevion.

Synthetic nanoparticle vaccines

At present there is considerable interest in synthetic nanoparticles (NP) as vaccine. Types of NPs include polymeric NPs, inorganic (non-biodegradable) NPs, ISCOMs (Immune Stimulating COMplexes), Liposomes, and self-assembling proteins like ferritin [79]. Non-recombinant spores of *Bacillus subtilis* as a mucosal vaccine delivery system has also been described [80]. A variety of biomaterials have/are being evaluated as vaccine delivery systems. Polymeric NPs are of this variety. Such particles have received considerable attention because they can co-deliver antigen and adjuvant either by encapsulation or chemical conjugation resulting in strong local innate immune activation, and they are biodegradable [81]. Polymeric NPs can be produced from different types of polymers namely poly(amino acids), polysaccharides or poly(α-hydroxy acids) [79, 82]. The most commonly used poly(α-hydroxy acids) for NP assembly is PLGA-poly(lactic-*co*-glycolic acid) or PLA-poly(lactic acid) [79, 82]. For vaccine induced immunity, the size of the NPs is clearly important, and this has been demonstrated experimentally [83]. The shape of NPs appears to be equally important in immune activation; surface textured (as opposed to smooth) polymeric NPs had a pronounced impact on immune cell uptake and inflammasome activation [84].

Several NP vaccine approaches have been used to produce RSV vaccine. In one study NPs were produced on 50 nm $CaCO_3$ core by alternately layering PGA (negatively charged poly glutamic acid) and PLL (positively charged poly-l-lysine). The outer most layer of this PGA-PLL seven layer film was the designed peptide (DP) containing RSV G protein CX3C motif connected to cationic sequence. This layer-by-layer (LbL) NP vaccine was injected into mice with 50 µg of DP/100 µl/ mouse two times, three weeks apart, and challenged intranasally with 10^6 PFU of RSV A2 strain. Mice produced robust IgG response and neutralizing antibodies and G protein- and M2-specific T cell responses that were protective in the lung following challenge [85, 86].

Gold NPs have also been used as a RSV vaccination strategy. Gold nanorods were formulated as a vaccine candidate by covalent conjugation of the nanorods to RSV F protein using a layer-by-layer approach. Vaccine potential of this approach was confirmed *in vitro* by demonstrating the induction of immune response in primary human T cells: Briefly, monocyte-derived human dendritic cells were exposed to this vaccine overnight at 37 ºC and then co-cultured the next day with CFSE-stained autologous T cells. Flow cytometry analysis demonstrated T cell proliferation [87].

CONCLUSION

The many human pathogens such as RSV, hPIV3, hMPV, and the newly emergent zoonotic paramyxovirus NiV belong to the family *Paramyxoviridae*. These viruses cause considerable morbidity and significant mortality and although lot of effort has, and is being expended, currently there is no licensed vaccine for any of these viruses. However, with the advent of newer vaccine technologies and better adjuvants, a clearer understanding of mechanisms of virus-host interactions, and the fact that vaccine-induced correlate of protection is already known to be neutralizing antibodies, there is every expectation that vaccines for these viruses should be possible. Particulate vaccine approaches such as VLPs, virosomes and synthetic NPs that mimic virus structure appear particularly promising. They are also potentially safe because they do not contain viral genome, and therefore they are not infectious. Native VLP-based vaccines for HPV are already commercially available. Gardasil (Merck & Co), effective against HPV types 6, 11, 16 and 18 was approved in 2006, and Cervarix (GSK), effective against oncogenic serotypes 16 and 18 was licensed in 2009.

CONFLICT OF INTEREST STATEMENT

None.

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