Published in final edited form as:

Nanomedicine (Lond). 2014 ; 9(12): 1857–1868. doi:10.2217/nnm.14.107.

Virus-like particles as antigenic nanomaterials for inducing protective immune responses in the lung

Agnieszka Rynda-Apple¹, Dustin P Patterson², and Trevor Douglas^{*,3}

¹Department of Microbiology & Immunology, Montana State University, Bozeman, MT, USA

²Department of Chemistry & Biochemistry, University of Texas at Tyler, Tyler, TX, USA

³Department of Chemistry, Indiana University, Bloomington, IN, USA

Abstract

The lung is a major entry point for many of the most detrimental pathogens to human health. The onslaught of pathogens encountered by the lung is counteracted by protective immune responses that are generated locally, which can be stimulated through vaccine strategies to prevent pathogen infections. Here, we discuss the use of virus-like particles (VLPs), nonpathogen derivatives of viruses or protein cage structures, to construct new vaccines exploiting the lung as a site for immunostimulation. VLPs are unique in their ability to be engineered with near molecular level detail and knowledge of their composition and structure. A summary of research in developing VLP-based vaccines for the lung is presented that suggests promising results for future vaccine development.

Keywords

antigens; biomaterials; iBALT; immunology; influenza; protein engineering; vaccine; virus-like particles

Nanoparticles present ideal sized scaffolds for attaching and presenting antigens to the immune system to induce responses in a multivalent presentation and that can also be taken up by cells. While there are many synthetic platforms that have been utilized, such as polymeric nanoparticles, dendrimers, and membranous structures (i.e., liposomes, polymerosomes and micelles) [1], in this review we will focus on the utilization of protein cages and/or virus-like particles (VLPs) as a class of nanoscaffolds that have great demonstrated potential. Several good reviews have been written on the topic of utilizing VLPs for development of vaccines and immunogenic nanomaterials [2–4], and in this review we want to provide a greater discussion on what makes VLPs so unique from a materials point of view. In addition, we will focus on utilizing VLPs for targeting protective immune

© 2014 Future Medicine Ltd

*Author for correspondence: trevdoug@indiana.edu.

Financial & competing interests disclosure

The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

responses in the respiratory tract (i.e., the lung), infections of which are the leading cause of illness in humans and deaths among children under the age of 5 years [5]. Our goal is to show how VLPs can be rationally manipulated, with structural and biochemical knowledge down to the molecular level, to construct designer antigenic nanoparticles for targeted protection against pathogens. In addition, we will outline how these have been utilized in eliciting immune responses in the lung, and where the potential impacts of the field are in the future (Figure 1).

Introduction to VLPs

VLPs are self-assembled protein architectures that, when devoid of any accessory proteins or nucleic acids, have a hollow interior surrounded by a multisubunit protein coat. In addition to classical VLPs that form well-defined, regular structures, another class of VLPs composed of an ensemble of biomolecules, but that are less regular in structure also exist [6]. Such particles are not the focus of discussion here, but will be noted in the case of influenza as background to VLPs being studied to combat this significant pathogen to human health. While in a strict sense VLPs are derived from viral components, other nonviral protein cage structures exist that resemble and have properties similar to VLPs. For simplicity we will refer to such nonviral protein cages as VLP like and throughout the review will predominantly use the VLP moniker in discussion of design, manipulation and immune responses, although the principles discussed apply to both classes. VLPs are nonpathogenic, and replication deficient with viral genomes and protein cofactors important for infectivity removed. This makes them ideally suited for constructing new vaccines, as there is no risk of virulence, which can occur with attenuated viral strains. In addition, they can be produced in high quantities through heterologous recombinant gene expression in a variety of expression systems, including *Escherichia coli*, yeast, baculovirus and mammalian cells. VLPs, due to their multicomponent structures and thus large molecular weights, are particularly simple to purify in many cases, making production on a large scale economically viable [7].

Because of extensive research in the field of virology and protein biochemistry, the structural and functional relationships that underpin a vast array of viruses and protein cages, can be exploited and utilized for modifying their VLP counterparts. The structural information is of particular significance since it allows the prediction with a large degree of certainty, at close to the molecular level, where all the atoms and amino acid side chains are located within the large macro-molecular VLP structure. This allows researchers to introduce changes to the molecular architectures with some level of knowledge and confidence for how these changes might modify the structure. This can be exploited by using site-selective mutagenesis to incorporate new amino acids, which are reactive or have other unique properties. For example, mutations to introduce a cysteine residue on the interior or exterior of the cage can allow a range of nonnative molecules, such as adjuvants or targeting moieties, to be covalently and selectively attached to the surface of choice [8]. While synthetic modifications are straight forward, they do not guarantee quantitative labeling. Genetic manipulation, by inserting peptides or proteins, guarantees exact placement and controlled stoichiometry of the added component (Figure 2). Genetic manipulation of these systems has included insertion of peptide sequences within flexible

loops on the exterior, or addition of peptides or proteins onto exposed termini of the VLP coat proteins. In addition, adapter proteins that associate with the capsid wall, either interior or exterior, can be genetically modified to incorporate large cargos on either the exterior or interior of the VLP. This ability to utilize both chemical and genetic approaches to modify VLPs, with their well-defined and homogeneous composition and structures, is unmatched by other nano systems that are typically employed for similar purposes. Furthermore, there are thousands of protein cages and viruses that have been characterized with a variety of sizes and physical properties, [9] providing a library of potential architectures from which to develop designer antigenic nanoparticles. Researchers in this field have only begun to scratch the surface in the ability to manipulate VLPs for multivalent display of antigens and other immunostimulatory agents and the achievements thus far, as discussed below, provide exciting possibilities for the future.

VLPs & induction of antigen-nonspecific protection

Mucosal surfaces for vaccine delivery

Over 90% of all infections are initiated at the mucosal surfaces of respiratory, GI or reproductive tracts. Respiratory infections are a common occurrence in individuals of all ages and immune status, and thus represent a significant global health concern. Despite significant efforts in the field of vaccine development, vaccines are still lacking against many clinically relevant lower respiratory tract pathogens. VLPs are known to induce potent mucosal immune responses presumably because they resemble viruses and have been shown to induce innate and adaptive immune responses [9,10]. Studies have shown that in order for VLPs to induce the most potent antibody responses, they must reach the lower parts of the lung, suggesting the importance of mucosa of the lung for the interaction between the VLPs and immune system [11].

Lung structure & function relationship Although resistance posed by the physical barriers prevents the vast majority of infections, some successful pathogens enter the body via either direct infection of mucosal epithelium or penetration of mucosal surfaces [9]. Unlike other mucosal sites within the body that are protected and immunologically shaped by the commensal flora, the lung has long been considered to be sterile, and only recently have researchers entertained the idea of commensal communities in the lung [12]. However, the network of commensal microorganisms in the lung is significantly under-represented when compared with other mucosal sites [12]. For these reasons the lung relies on an intricate network of immune cells and antimicrobial secretion for defense. The close relationship of structure (extensive vasculature) and function (gas exchange) in the lung makes this organ especially vulnerable to inflammation. However, this unique anatomy of the lung ensures its exposure to millions of external antigens on a daily basis. Thus, although execution of rapid and effective response to a pathogen is desired in the lung, protection of delicate lung tissue from inflammation-induced damage is essential for the proper function of this vital organ.

Formation of inducible bronchus-associated lymphoid tissue during immune response in the lung

To ensure rapid immune response, with simultaneous protection of lung tissue, local responses can be induced that are potent and minimally damaging. One example of the induction of an efficient yet nondamaging local immune response is the formation of bronchus-associated lymphoid tissue (BALT). Although constitutively present in some mammalian species, permanent BALT is not present in mice and humans [13,14]. However, formation of inducible BALT (iBALT) can occur in mice and humans in response to certain viral and bacterial infections, as well as sterile inflammation [15,16]. iBALT formation has been described in response to influenza infection in mice, and was reported to be sufficient for immune-mediated resolution of influenza infection, as mice lacking peripheral lymphoid organs (but possessing iBALT) not only cleared virus infection but also survived higher doses of virus challenge than normal mice [17]. Other results have supported the protective role of iBALT against various viral and bacterial infections, further suggesting that responses generated in iBALT are potentially less damaging to the lung tissue than systemic immune responses [17,18]. Intranasal inoculation of mice with small heat shock protein (sHSP) nanoparticles, largely due to formation of iBALT, protected mice against lethal challenges of various influenza viruses, pneumovirus, and even coronavirus responsible for severe acute respiratory syndrome (SARS-CoV) [18]. Moreover, sHSP inoculation significantly reduced morbidity associated with *Coxiella brunetii* infection, in contrast with saline-inoculated mice, and sHSP-inoculated mice retained their preinfection bodyweight despite having similar lung bacterial burdens. This further suggests that the sHSP-induced local immune response in the lung is less damaging than systemic immune response. Similar levels of protection were achieved after intranasal inoculation of mice with nanoparticles derived from the archaeon *Methanocaldococcus jannaschii* (sHSP) [18,19] or with P22 bacteriophage VLPs (P22-VLPs) [19] that carried no resemblance to each other or to the subsequent challenge agent, indicating that this VLP-induced protection was antigen-nonspecific.

The role of adaptive immune cells in the VLP-induced iBALT-mediated protection

As iBALT develops adjacent to the large airways and is known to contain B and T cells, follicular dendritic cells (FDCs) and germinal centers (GCs), mice lacking these adaptive cells have been shown to be incapable of developing iBALT in response to VLP inoculation [18]. Not only was the iBALT-mediated protection induced by various VLPs critically dependent on the presence of adaptive immune cells, but also the presence of preformed iBALT in mouse lungs prior to the challenge significantly reduced the inflammation-induced tissue damage otherwise associated with infection-induced iBALT [20]. Mice with preformed iBALT at the time of influenza A challenge not only had increased accumulation of alveolar macrophages (AMs) and DCs in their iBALT areas, but also in their tracheobronchial lymph nodes (TBLN), which translated into accelerated kinetics and intensified influenza-specific CD4 T cells responses in these mice [19]. Extensive toxicological studies have shown no adverse effects associated with multiple administrations of VLPs [18,19,21]. Moreover, formation of iBALT in either response to VLP treatment or infection of mice with the opportunistic fungus *Pneumocystis murina* [22] induced a type of

immune imprinting. Thus, targeted induction of iBALT by VLP-based vaccines would induce a state of immune readiness to a subsequent assault, which could potentially provide a broad range of protection against a variety of pathogens.

VLPs as potent inducers of humoral immunity

The majority of viruses are particles between 20–200 nm [23], which positions their size between small poorly immunogenic soluble particles, including proteins and small protein complexes often present in extracellular spaces (<10 nm), and bacteria that are much bigger and fall in the size range of >200–500 nm [23]. The size and particulate nature of viruses is commonly thought to be responsible for their ability to directly cross the wall of lymphatic vessels into the lymphatic system [24]. They are then thought to reach the B cell follicles in the lymph nodes on the surface of DCs, macrophages and B cells [11,23,25]. The highly repetitive protein subunit structure of viral capsids makes them well-equipped for induction of humoral immunity, due to efficient B-cell receptor (BcR) crosslinking and induction of neutralizing antibodies [11,23,25–28]. The ability to induce humoral immunity is advantageous for antiviral vaccines, because formation of pathogen-specific antibodies either directly prevents (in case of induction of neutralizing antibodies), or significantly accelerates (non-neutralizing antibodies) virus clearance [29]. As such, Link *et al.* demonstrated the importance of the repetitive structure of a viral capsid for induction of efficient adaptive immune responses. In an elegant study they reported that the repetitive structure of viral capsid was required for binding of natural (antigen nonspecific) IgM antibodies and fixing complement C1q complex for efficient activation of B cells and GC responses [25]. Systemic administration of Q β bacteriophage-derived VLPs (Q β -VLPs), but not soluble Q β -dimer, into naive mice resulted in efficient VLP binding to noncognate B cells via complement IgM and the VLPs were subsequently transported to FDCs, a process thought to be required for activation and clonal expansion of B cells within GCs [30,31]. This efficient transport of Q β -VLPs was independent of prior immunity, correlated with stronger induction of GC responses and was inhibited by the presence of Q β -specific IgG antibodies, which in turn were required for efficient induction of FDC responses by Q β -dimer. In another study, Bessa *et al.* demonstrated the importance of B cells as low-affinity transporters of VLPs from the lung mucosa to the spleen for induction of antibody responses. As induction of antibody responses occurs within B-cell follicles of secondary lymphoid organs rather than in mucosa, VLPs administered intranasally are bound by BcRs on lung B cells. This engagement of the BcR, in addition to the formation of local GCs and induction of VLP-specific IgA antibodies, resulted in the transport of BcR-bound VLPs into the splenic B cell follicles for induction of efficient systemic IgG responses [11]. It was also shown that the transport of VLPs by the B cells was independent of Fc receptor γ (FcR γ) or complement receptor 2 (CR2) and was limited to the low-affinity BcR.

Although the vast majority of vaccines are currently administered systemically, in an earlier study Bessa *et al.* demonstrated no differences in the efficacy of Q β -specific systemic IgG and IgA responses following subcutaneous versus intranasal immunization [10]. In contrast with the systemic immunization however, only intranasal immunization elicited local production of IgA. Results from our laboratory have shown that intranasal delivery of ovalbumin (OVA) chemically conjugated to sHSP derived from *Methanocaldococcus*

jannaschii (a hyperthermophilic archaeon) into naive mice significantly accelerated and intensified OVA-specific antibody responses when compared with mice inoculated with OVA alone, sHSP alone or with an OVA + sHSP admixture (where unconjugated OVA and sHSP were mixed together prior to inoculation of mice) [21]. In this case the sHSP was engineered, utilizing crystallographic structural information, to incorporate a reactive cysteine genetically via site-directed mutagenesis of the sHSP gene to allow covalent attachment and external display of the model OVA antigen. Conjugation of OVA was achieved by utilizing a heterobifunctional crosslinking reagent that reacted selectively with the cysteine thiol of the sHSP protein and lysine residues on OVA. Cysteine residue placement, as well as linker length within the heterobifunctional crosslinker, were screened to gain constructs that allowed optimal crosslinking of ovalbumin to the cage. The results from the optimized sHSP-OVA construct showed that conjugation yielded rapid production of antibodies against ovalbumin. In this regard, when OVA alone (unconjugated) was inoculated, a strong antibody response was not observed until day 14 postchallenge. However, conjugated sHSP-OVA yielded optimum IgG to OVA within 5 days after a single dose inoculation. In addition, the peak OVA-specific IgG titer occurs at least a day sooner, by day 4 postchallenge, by pretreatment of mice with a different VLP (derived from the bacteriophage P22). The enhancement of immunogenicity achieved by covalently attaching OVA to sHSP was attributed to a carrier effect and the immunogenicity observed by injecting OVA alone and VLP alone as an admixture was attributed to the adjuvant effects of VLPs. Moreover, pretreatment with P22-VLPs enhanced production of mucosal IgA, as well as an isotype-switched OVA-specific serum IgG. These results speak to the power of utilizing VLPs and the ability to manipulate structures chemically, which allows a broad range of antigens or other immunostimulatory molecules for constructing designer antigenic particles for directed immune responses.

VLP-based vaccines for influenza infection

Despite a significant worldwide effort into designing crossprotective influenza vaccines, influenza virus is one of the leading causes of respiratory distress, morbidity and even mortality worldwide. According to the WHO, seasonal influenza epidemics result in between 3 and 5 million cases of severe illnesses and >500,000 lethal cases per year [32,33]. The potential for vaccines targeting the respiratory mucosa has been acknowledged by US FDA-approved, intranasally delivered FluMist vaccine that was shown to be more effective in providing comprehensive local immunity than the injectable annual influenza vaccines [34,35]. Importantly, immunity induced by FluMist, but not its injectable counterpart, was shown to provide protection against secondary bacterial challenge of mice with *Streptococcus pneumoniae*, one of the leading causes of secondary post-influenza bacterial infections [36]. Thus, the environment of the respiratory mucosa makes it a desirable location for vaccine delivery.

Problems with current influenza vaccine formulations

Annual influenza outbreaks are difficult to control with the existing vaccines as these are formulated to induce neutralizing antibody responses to the specific subtypes of hemagglutinin (HA) and neuraminidase (NA), proteins on the virus capsid exterior that are

the most susceptible to amino acid mutation of antigenic sites, known as antigenic drift [37]. The additional problem associated with the current state of influenza vaccine development is an emerging number of avian-derived influenza A viruses (2006–2008 H5N1; 2013 H7N9, 2013 H10N8). It is estimated that wild birds are a source of 16 influenza HA subtypes to which the human immune system is naive [38–41]. Genetic reassortment of RNA between avian and human influenza and mutations can enable the virus to become transmissible among human population, which given the high virulence of avian source influenza may lead to global pandemic. As these avian influenza strains are highly pathogenic to domestic poultry, the current FDA approach for influenza vaccine production (i.e., raising in chicken eggs) may not be a viable option. To overcome this limitation, a number of non-egg raised VLP-based influenza vaccine platforms have been engineered and tested in mice and ferrets [42–44].

A major area of interest in the field of vaccine development for lung-associated pathogens like influenza is new vaccine strategies that will provide broad serotype protection. Currently FDA-approved formulations of the yearly influenza vaccines are a trivalent composite of two A strain and one B strain that have been determined to be the most prevalent in any given year. However, due to antigenic drift or shift, these vaccines often leave individuals susceptible to infection from new emerging strains of influenza not targeted by the vaccine formulations. In such a case, formulation and production of a supplementary vaccine in chicken eggs is costly, slow and may be ineffective if an emerging influenza strain is of an avian origin. As an alternative, VLPs provide a platform for rapid production and could be used to produce vaccine candidates against rapidly emerging strains. In addition, highly conserved antigens from influenza could be utilized to construct VLPs that provide broad serotype protection.

Influenza virus-based VLPs

Although influenza VLPs do not form regular structures, but are ensembles of influenza proteins, discussion here is warranted to provide a more complete picture of the strategies taken and limitations observed in combating this important pathogen to human health. Implemented as an alternative to the traditional influenza-vaccine design, structural proteins of influenza virus can be produced in mammalian cell lines and assembled into VLP particles and released into the cell culture media much like during the virus budding. This approach results in incorporation of virus glycoproteins into the surface of VLPs, and thus, influenza VLPs resemble the actual influenza morphologically [41]. A number of influenza VLPs have been generated in mammalian cell lines, or in insect cells using baculovirus expression system (described in detail in [41]). Animals intranasally vaccinated with these VLPs containing either HA or matrix protein (M1) of H3N2 influenza were protected from lethal infection with closely related H3N2 influenza [44]. This protection was based on induction of HA-specific neutralizing antibodies that were equally, if not more, protective than the sublethal vaccination with the corresponding live virus. In 2007, Quan *et al.* attempted to provide protection against heterologous influenza infection by immunization of mice with influenza VLPs containing M1 and HA proteins (M1-HA VLPs). Although improved survival was observed after heterologous influenza challenge, these VLPs were incomparably more protective against homologous challenge [43], and much like other

influenza-based VLPs were significantly more capable of inducing strong antibody responses than recombinant HA (rHA) alone [42,43]. Despite the reasonable success of VLP-based influenza vaccines, these were mostly protective against a parental (or closely related to the parental) strain and did not provide the broad serotype protection that is desired.

To overcome this strain specificity and to improve the overall immunogenicity of influenza VLP-based vaccines, immune adjuvants were incorporated in the VLP formulations [45-48]. For instance, inclusion of flagellin (TLR5 agonist) improved immunogenicity of influenza VLPs against heterosubtypic challenge [48]. As a safe alternative suited to a large-scale production, a plant-based expression system has been utilized for production of a VLP-based intramuscular vaccine that protected against $1 \times LD_{50}$ challenge with influenza in mice [49]. However, only a limited protection against mostly homologous or closely related heterologous strains of influenza was achieved with the use of these VLP-based systems.

VLPs not antigenically related to influenza as vehicles for influenza-specific vaccines

As noted in the previous section, VLPs derived from influenza can be obtained, but have not been shown to achieve broad crossprotection to heterologous influenza strains.

Alternatively, noninfluenza VLPs have been utilized as molecular scaffolds for displaying antigenic peptides and proteins from influenza to stimulate protective immune responses. This approach allows researchers to produce VLPs carrying additional influenza antigens that can be obtained through simple heterologous protein expression, and be produced in relatively high yields. In this section we will highlight some of the strategies that have been exploited for using noninfluenza VLPs as scaffolds for constructing influenza specific vaccines, which highlights some of the unique features for utilizing VLPs for vaccines.

As mentioned above, HA and NA proteins targeted by many influenza vaccine strategies are subject to an antigenic drift. As an alternative, a transmembrane M2 protein of influenza virus has been explored as a potential candidate for influenza vaccine [41]. Although M2 is a minor protein expressed by influenza virus, it forms a proton-selective ion channel, and thus it is essential for the productive viral infection. In contrast with HA and NA, the M2 protein is highly conserved across influenza species as it is not subject to antigenic drift or shift [37]. Furthermore, antibodies to M2 were shown to be protective against influenza infection in mice [50]. As a logical consequence, VLP-based vaccines targeting M2 have been engineered by multiple groups and tested for protection against influenza challenge. Utilizing the hepatitis B virus core protein (HBc) as a VLP scaffold, Neirynck *et al.* constructed a genetic fusion (i.e., splicing together two or more genes that will yield a fused polypeptide upon expression) of the extracellular domain of M2 and HBc. When tested *in vivo*, these M2-HBcVLPs protected mice against lethal challenge with influenza via induction of neutralizing antibodies [51]. Similar levels of protection were generated via either intranasal (mucosal) or intraperitoneal (systemic) administration of M2-HBc VLPs. However, multiple administrations of M2-HBc VLPs were required for protection lasting up to 26 weeks postimmunization.

Another VLP-based vaccine against influenza was engineered by chemical coupling of M2 immunodominant region to Q β bacteriophage [52]. M2-Q β -VLPs induced protection against

influenza challenge that was mediated by antibody-dependent cell-mediated cyto-toxicity. M2-specific antibodies induced by M2-Q β -administration ameliorated disease caused by low but not high challenge doses of influenza virus, and due to the nature of the antibody-dependent cell-mediated cytotoxicity response, did not prevent influenza infection, making it insufficient during yearly epidemics, where full protection is desirable.

Genetic fusion of the universal M2e epitope to Papaya mosaic virus (PapMV) VLPs led to the production of anti-M2e antibodies, and when coadministered with alum provided nearly complete protection against lethal challenge of mice with influenza [53]. Interestingly, the immunogenicity of the PapMV vaccine platform was critically dependent on multimerization of the coat protein, and monomeric PapMV fused to a hepatitis C virus epitope was insufficient for induction of immune response to either the platform of the fused epitope [54]. In another study by the same group, PapMV VLPs were used as an adjuvant to improve efficacy of the trivalent human inactivated influenza (TIV) vaccine [55]. When administered systemically PapMV VLPs enhanced immune response to co-administered TIV and provided protection against challenge with heterosubtypic influenza virus. Protection against lethal influenza challenge of mice can be generated by intranasal administration of VLPs that not only show no biochemical resemblance to influenza virus, but also contain no influenza epitopes [18,22]. Mice inoculated with either sHSP or the P22 VLP were shown to be significantly more resistant to H1N1 influenza challenge, and when compared with saline-inoculated mice VLP-protected mice had reduced lung viral titers, did not lose body weight, and had improved survival rates.

A somewhat unconventional approach to antigen display has been described utilizing VLP from the bacteriophage P22, where the interior space of the P22 capsid was utilized for placement of influenza antigen [56]. In this case, the well-characterized self-assembly of P22 was exploited, which is coordinated by scaffolding proteins (SPs) that direct the assembly of 420 CP into a T = 7 VLP, where T represents the triangulation number describing the virus structure. In a biomimetic strategy the conserved nucleoprotein (NP) was incorporated on the interior of P22, producing a VLP with NP displayed as it would be in influenza, on the interior. Incorporation of NP was achieved by creating a fusion protein of NP and the scaffolding protein (SP) of P22, which directs assembly of the P22 coat protein resulting in incorporation of the NP-SP on the interior of the VLP. The approach showed that particles containing high internal concentrations of either the NP-SP fusion or truncated NP variants were easily engineered, yielding homogeneous P22 VLPs containing NP that were rapidly purified in only a few steps. NP-P22 VLPs provided exquisite protection against high doses, 50–100 LD₅₀, of multiple serotypes of influenza. In addition, protection was found to be CD8 T-cell dependent, which aids in clearance of infected cells that express nonexposed (internal) influenza peptides such as those from influenza NP. Interestingly, it was also observed that an antibody response was only found when full length NP was encapsulated, which was not observed for a truncated variant NP that showed the same CD8 T-cell-dependent level of protection. These results suggest that by engineering the VLPs and controlling where antigens are displayed, either antibody- or CD8 T-cell-mediated responses can be targeted.

Using VLPs derived from flockhouse virus (FHV), researchers have incorporated peptides into the flexible loop regions of the VLP, derived from the conserved stalk portion of HA and composing the major binding surface for the broadly protective antibody CR6261 [57]. They hypothesized that by displaying these smaller conserved fragments on a VLP platform, the broadly protective antibody against the fragment of the HA stalk could be generated to produce a broad serotype protective response. In their design, they incorporated a helix–turn–helix peptide containing, in part, the conserved peptide that provided the major binding surface for the neutralizing antibody CR6261. They had predicted that the helix–turn–helix peptide motif would retain the helical structure of the peptide epitope, providing the native structure of the peptide as found in the full length HA when inserted into the flexible loop region exposed on the surface of FHV. While this is a promising strategy based on protein structure, particles of FHV containing the prescribed peptide fragment were unable to elicit neutralizing antibody responses. Cryo-EM reconstructions revealed that of the two different constructs made, the construct lacking the desired helical peptide structure yielded the best protection in comparison to the construct that was found to maintain a helix–turn–helix motif that maintained the structural context found for the peptide within full length HA. This lack of a protective immune response by the well-folded helix–turn–helix construct has been suggested to result from a sterically hindered orientation of the peptide fragments displayed within the loops on the FHV. While re-engineering the particles to allow for better exposure of the peptides may provide the intended immune response, this strategy still provides a useful starting point for future work in the area of vaccine development.

More recently Nabel and coworkers have constructed immunogenic nanoparticles utilizing the protein cage nanoparticle ferritin as a VLP-like platform for the display of full length HA [58]. Structural information on ferritin (Fn) indicated that the N-terminus of the Fn VLP projected toward the exterior and that the cage subunits are organized in a threefold arrangement that spatially complemented that of the trimeric HA stalk (at the C-terminus of HA). Using this structural approach a fusion protein consisting of full length HA with its C-terminus fused to the N-terminus of the Fn subunit was constructed. Recombinant mammalian cell expression of the HA-Fn fusion yielded what appeared to be fully assembled VLPs consisting of 8 protruding HAs. *In vivo* studies with mice and ferrets showed that the HA-Fn VLPs produced a more potent immune response and broader protection than currently licensed seasonal flu vaccines. These findings indicate the potential power of the VLP engineering approach and highlight the unique ability to combine molecular/structural information from crystallography and cryo-electron microscopy with rational design and bioengineering of immunogenic nanoparticles.

VLPs& induction of DC responses

One of the mechanisms of self versus non-self recognition is mediated by pattern recognition receptors (PRRs) abundantly expressed on DCs, as well as mucosal epithelium [59-64]. PRRs recognize highly conserved structures among pathogens termed pathogen-associated molecular patterns (PAMP) [65] and distinguish them from patterns of host cellular damage, called danger-associated molecular patterns [66]. While PRR stimulation by PAMPs generally results in pathogen-induced inflammation and associated tissue damage, danger-associated molecular pattern-induced sterile inflammation tends to be much

less damaging to the host [65,66]. Thus, it has been recognized that the ability to prime the immune system for induction of nondamaging inflammation could be beneficial for the development of immuno-prophylaxes and immunotherapeutics. PRR targeting as a means of amplifying and focusing VLP-induced immune has been explored by multiple groups. Bessa *et al.* demonstrated that Q β VLPs spontaneously package bacterial RNA during self-assembly process, and thus carry ligands for TLR7 and TLR3 that are efficient inducers of mucosal and systemic immune responses [10]. The same group more recently reported that unlike soluble protein antigen (in that case – Q β dimer), Q β VLPs, presumably due to their size and repetitive structure, are efficient inducers of FDCs, a phenomenon that directly translates into efficient antigen presentation for B-cell activation [25]. The authors presented a compelling argument for more efficient transport of VLPs to B-cell follicles, which correlated with stronger induction of GCs. In another elegant study this same group demonstrated that only the CD8⁺ subset of DCs is responsible for the presentation of VLPs in the context of the CD8 T cells (MHC class I pathway), yet the presentation of VLPs utilizing the MHC class II pathway (to CD4 T cells) possess no such restriction, and in fact can be performed by either CD8⁺ and CD8[−] subsets of DCs [67].

An antigen presentation in the context of the lung is predominantly accomplished by either CD11c⁺ alveolar macrophages (AMs) or DCs (airway CD103⁺ DCs and parenchymal CD11b⁺ DCs). It has recently been shown that the intranasally delivered VLP accelerates maturation and trafficking of antigen presenting cells in the CCR2-dependent manner [22]. Interestingly we have shown that a similar effect on lung CD11c⁺ cells can be elicited by infection of mice with the opportunistic fungus, *Pneumocystis murina*, and as such either infection with *Pneumocystis* or VLP inoculation-protected mice from a challenge with lethal doses of influenza virus. Therefore, nasal installation of VLPs immunologically imprints the lung environment making it more reactive and less damaging in the context of the subsequent infections.

VLP-induced protection from bacterial pneumonia: role for the innate immunity

Intranasal inoculation of mice with VLPs can also protect them from pulmonary challenge with bacterial pathogens. Mice instilled with five doses of sHSP were shown to be protected from morbidity following *Coxiella burnetii* challenge [18]. Although the nanoparticles did not reduce lung bacterial burdens of *Coxiella*, in contrast with mock-treated mice, nanoparticle-protected mice did not lose bodyweight as a result of *Coxiella* infection. In a more recent study, it was demonstrated that mice treated with sHSP are significantly more resistant to lethal pulmonary challenge with methicillin-resistant *Staphylococcus aureus* [68]. Interestingly, unlike sHSP-mediated protection from influenza virus, this was largely due to an innate immune response. In this regard, both SCID and WT mice treated with sHSP were equally protected from methicillin-resistant *Staphylococcus aureus* pneumonia, suggesting an important role for the innate immune system, more specifically CD11c⁺ cells and neutrophils in sHSP-mediated protection from gram positive bacterium. Our results were confirmed in a recent study demonstrating that protection from pulmonary challenge with either influenza virus or *Streptococcus pneumoniae* can be achieved in mice inoculated

with PapMV VLPs [65,69]. Also in agreement with our results, stimulation of innate immune signaling and reduced lung tissue damage was suggested to be responsible for the protection following PapMV VLPs inoculation. Thus, VLPs represent a unique and versatile strategy for alteration of the immune response in the lung, which combined with the VLP-mediated reduction of the inflammation-mediated host tissue damage makes them ideal candidates for vaccine platforms against a variety of lung pathogens.

Conclusion & future perspective

Utilizing VLPs for stimulating protective immune responses has been well reported and commercial vaccines based on VLPs are clinically available. However, the use of VLPs as scaffolds that can be manipulated and engineered, with near atomic level precision, to incorporate antigens or immunostimulative molecules of choice for multivalent display provides even greater prospects for advanced vaccine development. It is highly desirable to design, engineer and produce particles by completely genetic means, which allows for rapid manufacture of the complete VLP–antigen complex through heterologous expression in a range of host cells. VLP platforms permit display of immunogenic materials on both the inside and the outside of the capsid, which could be used for selective display and to direct immune responses based on the spatial arrangement of antigens. In addition, harboring multiple kinds of immunostimulative and targeting moieties on the same VLP platform could result in a construct containing for example a protein antigen with an adjuvant. This approach would allow for co-display within the context of the same nanoparticle. Utilizing VLPs allows for modifications to be made with near molecular level precision, which is not easily accomplished with other nanoparticle platforms. In addition, the ability of VLPs to generate protective immune responses in the lung is particularly important because of the prevalence and potential for future epidemics caused by respiratory pathogens. Some targets that are of particular interest include influenza, which current vaccine strategies cannot fully protect against. Other respiratory pathogens such as respiratory syncytial virus (RSV), to which a vaccine is not currently available, could also be targeted by VLP technology, which has the potential to overcome limitations associated with classical vaccine design.

Acknowledgments

This work was supported in part by a grant from the NIH, NIAID R01-AI104905.

References

1. Smith DM, Simon JK, Baker JR. Applications of nanotechnology for immunology. *Nat. Rev. Immunol.* 2013; 13(8):592–605. [PubMed: 23883969]
2. Grgacic EVL, Anderson DA. Virus-like particles: passport to immune recognition. *Methods.* 2006; 40(1):60–65. [PubMed: 16997714]
3. Plummer EM, Manchester M. Viral nanoparticles and virus-like particles: platforms for contemporary vaccine design. *Wires Nanomed. Nanobi.* 2011; 3(2):174–196.
4. Schneider-Ohrum K, Ross TM. Virus-like particles for antigen delivery at mucosal surfaces. *Curr. Top. Microbiol.* 2012; 354:53–73.
5. Fendrick AM, Monto AS, Nightengale B, Sarnes M. The economic burden of non-influenza-related viral respiratory tract infection in the United States. *Arch. Intern. Med.* 2003; 163(4):487–494. [PubMed: 12588210]

6. Pattenden LK, Middelberg APJ, Niebert M, Lipin DI. Towards the preparative and large-scale precision manufacture of virus-like particles. *Trends Biotechnol.* 2005; 23(10):523–529. [PubMed: 16084615]
7. Roldao A, Mellado MCM, Castilho LR, Carrondo MJT, Alves PM. Virus-like particles in vaccine development. *Expert Rev. Vaccines.* 2010; 9(10):1149–1176. [PubMed: 20923267]
8. Uchida M, Klem MT, Allen M, et al. Biological containers: protein cages as multifunctional nanoplatfoms. *Adv. Mater.* 2007; 19(8):1025–1042.
9. Neutra MR, Kozlowski PA. Mucosal vaccines: the promise and the challenge. *Nat. Rev. Immunol.* 2006; 6(2):148–158. [PubMed: 16491139]
10. Bessa J, Schmitz N, Hinton HJ, Schwarz K, Jegerlehner A, Bachmann MF. Efficient induction of mucosal and systemic immune responses by virus-like particles administered intranasally: implications for vaccine design. *Eur. J. Immunol.* 2008; 38(1):114–126. [PubMed: 18081037]
11. Bessa J, Zabel F, Link A, et al. Low-affinity B cells transport viral particles from the lung to the spleen to initiate antibody responses. *Proc. Natl Acad. Sci. USA.* 2012; 109(50):20566–20571. [PubMed: 23169669]
12. Ichinohe T, Pang IK, Kumamoto Y, et al. Microbiota regulates immune defense against respiratory tract influenza A virus infection. *Proc. Natl Acad. Sci. USA.* 2011; 108(13):5354–5359. [PubMed: 21402903]
13. Pabst R. Is BALT a major component of the human lung immune system? *Immunol Today.* 1992; 13(4):119–122. [PubMed: 1580992]
14. Pabst R, Gehrke I. Is the bronchus-associated lymphoid tissue (BALT) an integral structure of the lung in normal mammals, including humans? *Am. J. Respir. Cell Mol. Biol.* 1990; 3(2):131–135. [PubMed: 2378747]
15. Tschernig T, Pabst R. Bronchus-associated lymphoid tissue (BALT) is not present in the normal adult lung but in different diseases. *Pathobiology.* 2000; 68(1):1–8. [PubMed: 10859525]
16. Randall TD. Bronchus-associated lymphoid tissue (BALT) structure and function. *Adv. Immunol.* 2010; 107:187–241. [PubMed: 21034975]
17. Moyron-Quiroz JE, Rangel-Moreno J, Kusser K, et al. Role of inducible bronchus associated lymphoid tissue (iBALT) in respiratory immunity. *Nat. Med.* 2004; 10(9):927–934. [PubMed: 15311275]
18. Wiley JA, Richert LE, Swain SD, et al. Inducible bronchus-associated lymphoid tissue elicited by a protein cage nanoparticle enhances protection in mice against diverse respiratory viruses. *PLoS One.* 2009; 4(9):e7142. [PubMed: 19774076]
19. Richert LE, Hamsen AL, Rynda-Apple A, Wiley JA, Douglas T, Harmsen AG. Inducible bronchus-associated lymphoid tissue (iBALT) synergizes with local lymph nodes during antiviral CD4⁺ T cell responses. *Lymphat. Res. Biol.* 2013; 11(4):196–202. [PubMed: 24364842]
20. Jupelli M, Shimada K, Chiba N, et al. Chlamydia pneumoniae infection in mice induces chronic lung inflammation, iBALT formation, and fibrosis. *PLoS One.* 2013; 8(10):e77447. [PubMed: 24204830]
21. Richert LE, Servid AE, Harmsen AL, et al. A virus-like particle vaccine platform elicits heightened and hastened local lung mucosal antibody production after a single dose. *Vaccine.* 2012; 30(24):3653–3665. [PubMed: 22465748]
22. Richert LE, Rynda-Apple A, Harmsen AL, et al. CD11c⁺ cells primed with unrelated antigen facilitate an accelerated immune responses to influenza virus in mice. *Eur. J. Immunol.* 2013; 44:397–408. [PubMed: 24222381]
23. Zabel F, Kundig TM, Bachmann MF. Virus-induced humoral immunity: on how B cell responses are initiated. *Curr. Opin Virol.* 2013; 3(3):357–362. [PubMed: 23731601]
24. Manolova V, Flace A, Bauer M, Schwarz K, Saudan P, Bachmann MF. Nanoparticles target distinct dendritic cell populations according to their size. *Eur. J. Immunol.* 2008; 38(5):1404–1413. [PubMed: 18389478]
25. Link A, Zabel F, Schnetzler Y, Titz A, Brombacher F, Bachmann MF. Innate immunity mediates follicular transport of particulate but not soluble protein antigen. *J. Immunol.* 2012; 188(8):3724–3733. [PubMed: 22427639]

26. Lechner F, Jegerlehner A, Tissot AC, et al. Virus-like particles as a modular system for novel vaccines. *Intervirology*. 2002; 45(4–6):212–217. [PubMed: 12566703]
27. Ruedl C, Schwarz K, Jegerlehner A, Storni T, Manolova V, Bachmann MF. Virus-like particles as carriers for T-cell epitopes: limited inhibition of T-cell priming by carrier-specific antibodies. *J. Virol.* 2005; 79(2):717–724. [PubMed: 15613299]
28. Bachmann MF, Zinkernagel RM. Neutralizing antiviral B cell responses. *Annu. Rev. Immunol.* 1997; 15:235–270. [PubMed: 9143688]
29. Bachmann MF, Kopf M. The role of B cells in acute and chronic infections. *Curr. Opin Immunol.* 1999; 11(3):332–339. [PubMed: 10375561]
30. Carroll MC. The complement system in regulation of adaptive immunity. *Nat. Immunol.* 2004; 5(10):981–986. [PubMed: 15454921]
31. Carroll MC. The complement system in B cell regulation. *Mol. Immunol.* 2004; 41(2–3):141–146. [PubMed: 15159059]
32. Kalbfuss B, Knochlein A, Krober T, Reichl U. Monitoring influenza virus content in vaccine production: precise assays for the quantitation of hemagglutination and neuraminidase activity. *Biologicals*. 2008; 36(3):145–161. [PubMed: 18561375]
33. Thompson CM, Petiot E, Lennaert A, Henry O, Kamen AA. Analytical technologies for influenza virus-like particle candidate vaccines: challenges and emerging approaches. *Virol. J.* 2013; 10:141. [PubMed: 23642219]
34. Cox RJ, Brokstad KA, Ogra P. Influenza virus: immunity and vaccination strategies. Comparison of the immune response to inactivated and live, attenuated influenza vaccines. *Scand. J. Immunol.* 2004; 59(1):1–15.
35. Belshe R, Lee MS, Walker RE, Stoddard J, Mendelman PM. Safety, immunogenicity and efficacy of intranasal, live attenuated influenza vaccine. *Expert Rev. Vaccines*. 2004; 3(6):643–654. [PubMed: 15606348]
36. Sun K, Ye J, Perez DR, Metzger DW. Seasonal FluMist vaccination induces cross-reactive T cell immunity against H1N1 (2009) influenza and secondary bacterial infections. *J. Immunol.* 2011; 186(2):987–993. [PubMed: 21160043]
37. Kaiser J. A One-size-fits-all flu vaccine? *Science*. 2006; 312(5772):380–382. [PubMed: 16627732]
38. Fouchier RA, Munster V, Wallensten A, et al. Characterization of a novel influenza A virus hemagglutinin subtype (H16) obtained from black-headed gulls. *J. Virol.* 2005; 79(5):2814–2822. [PubMed: 15709000]
39. Rohm C, Zhou N, Suss J, Mackenzie J, Webster RG. Characterization of a novel influenza hemagglutinin, H15: criteria for determination of influenza A subtypes. *Virology*. 1996; 217(2): 508–516. [PubMed: 8610442]
40. Webster RG, Bean WJ, Gorman OT, Chambers TM, Kawaoka Y. Evolution and ecology of influenza A viruses. *Microbiol. Rev.* 1992; 56(1):152–179. [PubMed: 1579108]
41. Kang SM, Song JM, Quan FS, Compans RW. Influenza vaccines based on virus-like particles. *Virus Res.* 2009; 143(2):140–146. [PubMed: 19374929]
42. Bright RA, Carter DM, Daniluk S, et al. Influenza virus-like particles elicit broader immune responses than whole virion inactivated influenza virus or recombinant hemagglutinin. *Vaccine*. 2007; 25(19):3871–3878. [PubMed: 17337102]
43. Quan FS, Huang C, Compans RW, Kang SM. Virus-like particle vaccine induces protective immunity against homologous and heterologous strains of influenza virus. *J. Virol.* 2007; 81(7): 3514–3524. [PubMed: 17251294]
44. Galarza JM, Latham T, Cupo A. Virus-like particle (VLP) vaccine conferred complete protection against a lethal influenza virus challenge. *Viral Immunol.* 2005; 18(1):244–251. [PubMed: 15802970]
45. Quan FS, Compans RW, Nguyen HH, Kang SM. Induction of heterosubtypic immunity to influenza virus by intranasal immunization. *J. Virol.* 2008; 82(3):1350–1359. [PubMed: 18032492]
46. Tumpey TM, Renshaw M, Clements JD, Katz JM. Mucosal delivery of inactivated influenza vaccine induces B-cell-dependent heterosubtypic cross-protection against lethal influenza A H5N1 virus infection. *J. Virol.* 2001; 75(11):5141–5150. [PubMed: 11333895]

47. Skountzou I, Quan FS, Gangadhara S, et al. Incorporation of glycosylphosphatidylinositol-anchored granulocyte-macrophage colony-stimulating factor or CD40 ligand enhances immunogenicity of chimeric simian immunodeficiency virus-like particles. *J. Virol.* 2007; 81(3): 1083–1094. [PubMed: 17108046]
48. Wang BZ, Quan FS, Kang SM, Bozja J, Skountzou I, Compans RW. Incorporation of membrane-anchored flagellin into influenza virus-like particles enhances the breadth of immune responses. *J. Virol.* 2008; 82(23):11813–11823. [PubMed: 18786995]
49. D'aoust MA, Lavoie PO, Couture MM, et al. Influenza virus-like particles produced by transient expression in *Nicotiana benthamiana* induce a protective immune response against a lethal viral challenge in mice. *Plant Biotechnol. J.* 2008; 6(9):930–940. [PubMed: 19076615]
50. Treanor JJ, Tierney EL, Zebedee SL, Lamb RA, Murphy BR. Passively transferred monoclonal antibody to the M2 protein inhibits influenza A virus replication in mice. *J. Virol.* 1990; 64(3):1375–1377. [PubMed: 2304147]
51. Neiryneck S, Deroo T, Saelens X, Vanlandschoot P, Jou WM, Fiers W. A universal influenza A vaccine based on the extracellular domain of the M2 protein. *Nat. Med.* 1999; 5(10):1157–1163. [PubMed: 10502819]
52. Jegerlehner A, Schmitz N, Storni T, Bachmann MF. Influenza A vaccine based on the extracellular domain of M2: weak protection mediated via antibody-dependent NK cell activity. *J. Immunol.* 2004; 172(9):5598–5605. [PubMed: 15100303]
53. Denis J, Acosta-Ramirez E, Zhao Y, et al. Development of a universal influenza A vaccine based on the M2e peptide fused to the papaya mosaic virus (PapMV) vaccine platform. *Vaccine.* 2008; 26(27–28):3395–3403. [PubMed: 18511159]
54. Denis J, Acosta-Ramirez E, Savard C, et al. Immunogenicity of papaya mosaic virus-like particles fused to a hepatitis C virus epitope: evidence for the critical function of multimerization. *Virology.* 2007; 363(1):59–68. [PubMed: 17320136]
55. Savard C, Guérin A, Drouin K, et al. Improvement of the trivalent inactivated flu vaccine using PapMV nanoparticles. *PLoS ONE.* 2011; 6(6):e21522. [PubMed: 21747909]
56. Patterson DP, Rynda-Apple A, Harmsen AL, Harmsen AG, Douglas T. Biomimetic antigenic nanoparticles elicit controlled protective immune response to influenza. *ACS Nano.* 2013; 7(4): 3036–3044. [PubMed: 23540530]
57. Schneemann A, Speir JA, Tan GS, et al. A virus-like particle that elicits cross-reactive antibodies to the conserved stem of influenza virus hemagglutinin. *J. Virol.* 2012; 86(21):11686–11697. [PubMed: 22896619]
58. Kanekiyo M, Wei CJ, Yassine HM, et al. Self-assembling influenza nanoparticle vaccines elicit broadly neutralizing H1N1 antibodies. *Nature.* 2013; 499(7456):102–106. [PubMed: 23698367]
59. Greene CM, McElvaney NG. Toll-like receptor expression and function in airway epithelial cells. *Arch. Immunol. Ther. Exp. (Warsz).* 2005; 53(5):418–427. [PubMed: 16314825]
60. Akira S, Yamamoto M, Takeda K. Role of adapters in Toll-like receptor signalling. *Biochem. Soc. Trans.* 2003; 31:637–642. Pt 3. [PubMed: 12773172]
61. Takeda K, Kaisho T, Akira S. Toll-like receptors. *Annu. Rev. Immunol.* 2003; 21:335–376. [PubMed: 12524386]
62. Van Kooyk Y. C-type lectins on dendritic cells: key modulators for the induction of immune responses. *Biochem. Soc. Trans.* 2008; 36:1478–1481. Pt 6. [PubMed: 19021579]
63. Canton J, Neculai D, Grinstein S. Scavenger receptors in homeostasis and immunity. *Nat. Rev. Immunol.* 2013; 13(9):621–634. [PubMed: 23928573]
64. Palm NW, Medzhitov R. Pattern recognition receptors and control of adaptive immunity. *Immunol. Rev.* 2009; 227(1):221–233. [PubMed: 19120487]
65. Iwasaki A, Medzhitov R. Regulation of adaptive immunity by the innate immune system. *Science.* 2010; 327(5963):291–295. [PubMed: 20075244]
66. Chen GY, Nunez G. Sterile inflammation: sensing and reacting to damage. *Nat. Rev. Immunol.* 2010; 10(12):826–837. [PubMed: 21088683]
67. Keller SA, Bauer M, Manolova V, Muntwiler S, Saudan P, Bachmann MF. Cutting edge: limited specialization of dendritic cell subsets for MHC class II-associated presentation of viral particles. *J. Immunol.* 2010; 184(1):26–29. [PubMed: 19949081]

68. Rynda-Apple A, Dobrinen E, Mcalpine M, et al. Virus-like particle-induced protection against MRSA pneumonia is dependent on IL-13 and enhancement of phagocyte function. *Am. J. Pathol.* 2012; 181(1):196–210. [PubMed: 22642909]
69. Mathieu C, Rioux G, Dumas MC, Leclerc D. Induction of innate immunity in lungs with virus-like nanoparticles leads to protection against influenza and *Streptococcus pneumoniae* challenge. *Nanomedicine.* 2013; 9(7):839–848. [PubMed: 23499666]

Executive summary

Introduction to virus-like particles

- Virus-like particles (VLPs) are self assembled protein structures often containing hollow interiors.
- VLPs can be produced heterologously through different hosts that allow rapid generation and cost-effective production.
- Biochemical and structural knowledge of viruses allow manipulation of VLPs with near atomic level knowledge.
- Chemical and/or genetic approaches can be utilized to incorporate antigens or other immunostimulative moieties onto VLPs.
- VLPs serve as a platform for immunostimulative materials development.

VLPs & induction of antigen-nonspecific protection

- Mucosal surfaces are a predominant entry point of pathogens that cause severe human disease.
- VLPs induce potent mucosal immune responses.
- VLPs can be used to stimulate inducible bronchus-associated lymphoid tissue, which provides local immune protection in the lung.
- Inducible bronchus-associated lymphoid tissue provides rapid immune responses against pathogens in the lung.

VLPs as potent inducers of humoral immunity

- VLPs size and repetitive structure make them ideal platforms for inducing humoral immunity.

VLP-based vaccines for influenza infection

- Influenza presents a constant threat to human health and new vaccine strategies are needed to provide broad protection.
- VLPs derived from influenza provide limited protection and are not suitable for vaccine development.
- VLPs derived from noninfluenza virus present useful scaffold for influenza vaccine development.
- Molecular engineering of VLPs has provided great promise for future development of influenza vaccines.

VLPs & induction of dendritic cell responses

- Repetitive structures of VLP scaffolds can be exploited for induction of dendritic cell responses through pattern recognition receptors.
- VLP-induced protection from bacterial pneumonia: role for the innate immunity

- VLPs can provide protection against secondary bacterial infections of the lung.

Figure 1. Virus-like particles prime the local lung environment for efficient immune responses to a subsequent viral or bacterial challenge

Administration of VLPs enhances activity of lung resident CD11c+ cells and recruited neutrophils in an antigen-nonspecific manner. Enhancement of maturation and trafficking of CD11c+ cells results in accelerated humoral responses to influenza virus challenge. iBALT formation resulting from intranasal VLP administration enhances clearance of influenza virus by CD4+ T cells and antibodies with simultaneous inhibition of inflammation-induced lung tissue damage. Clearance of bacterial pathogens like MRSA occurs via VLP-mediated enhancement of bactericidal activity of innate cells including neutrophils and CD11c+ cells. FDC: Follicular dendritic cell; iBALT: Inducible bronchus-associated lymphoid tissue; MRSA: Methicillin-resistant *Staphylococcus aureus*; VLP: Virus-like particle.

Figure 2. Genetic manipulation of virus-like particles

Virus-like particles can be genetically modified to incorporate peptide and protein antigens by genetic fusion to the C/N-termini (top), scaffolding or other protein adapters that associate with the protein capsid (middle), or by insertion of peptide sequences with the flexible loop regions of the capsid. Genetic modification, shown schematically as modifications to rectangles representing the gene segments, ensures exact placement within the final virus-like particle structure and provides exact knowledge of the copies found within each particles.