

*Review Article***Virus-like Nanoparticles: Display Matters**

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An efficacious way to avoid atleast a bunch of infections by microbes is to neutralize the microbial infection right at the very beginning. This is well achieved by vaccination against many infectious agents. While vaccine is available for many pathogenic micro-organisms, we are still struggling to achieve success to develop prophylactic strategies for many others. Most commonly, the vaccines are developed by attenuation/ inactivation of infectious agents or by using killed pathogens. Yet another variety includes the sub-unit vaccines. In this review, a subcategory of subunit vaccine has been discussed; where the viral surface antigens when displayed in a rigid multivalent fashion at high densities, induce very potent and robust neutralizing antibody response as a result of very strong and extensive cross-linking of B-cell receptors.

Keywords: Nanoparticles; VLP; Rigid; Multivalent; High-density; Immunogen

Introduction

A vaccine is a formulation that would provide active immunity against a particular infectious disease. The most common and well accepted vaccine categories for viral diseases include the live attenuated and the killed pathogens. These involve use of the whole virus that still has its genome intact and has been either grown in non-human culture system to attenuate the virus, thus making it less pathogenic in humans OR, converted to a non-pathogenic form by various treatments. For many years, these types of vaccines have prevented us from an array of difficult viral infections like small pox, polio (Sabin and Salk) etc. While live attenuated vaccines are better immunogens, the ones that are killed imparts less durable resistance and require multiple boosters. A third category of vaccines involve the sub-unit vaccines (SUVs) and are also comparatively less immunogenic.

A separate class of viral vaccines involve Virus-LIKE-Particles (VLPs) (20-60nm in diameter) and is an advancement of the SUVs. VLPs are basically nothing but a virus devoid of genetic material and thus replication defective. However, a VLP with rigidly multivalent display of viral surface proteins are

extremely immunogenic and induce exceptionally potent neutralizing antibody response in immunized individuals. The rigidity, correct conformation and specially the highly repetitive array of viral surface proteins lead to a better cross-linking of B-cell immunoglobulin receptors and thus better B-cell activation, as opposed vaccine candidates involving isolated viral surface proteins or derivatives, thereof.

Journey of a Virus in the Body and Immune Response

Viruses, upon release in the human body can follow two basic routes to reach the lymph nodes. Viruses smaller in size can freely traffic through the lymphatic system and zigzag its way to the lymph nodes, whereas other viruses are taken up by the cells of the immune system like; B cells, dendritic cells and the macrophages and also viral antigens are transported to the lymph nodes. In the lymph nodes adaptive immune responses are induced. In a lymph node, the viral antigens are presented by follicular dendritic cells to the B cells. Upon recognition of surface specific antigens by B-cell receptors (BCRs), B-cell activation occurs, followed by further presentation by MHC molecules triggering T cells.

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VLPs: Rigidly Multivalent Repetitive High Density Display of Antigens

In 1993, Bachmann *et al.* published in their Science paper that organization of antigens influence B cell tolerance. They showed that B cells recognize antigens displayed with repetitive 50-100Å spacing in a distinctive manner to induce potent activation signals. B-cells have evolved to recognize pathogen associated molecular patterns (PAMPs) by engaging the B cell receptors. Repetitive 50-100Å spaced antigens can be categorized as a type of PAMP and are common on viral surfaces, but not so common in case of vertebrate self-antigens (Bachmann and Zinkernagel, 1996). B-cell receptor clustering due to this type of antigen spacing leads to exceptionally strong downstream activation and survival signals and high titer neutralizing antibody production. It has been shown that high density virus like display of antigens with 50-100Å spacing can even break the self-tolerance (Chakerian *et al.*, 2002) and that possibly, the immune system has evolved to accept this positively, because of the fact that this type of high density display is not so common in case of self antigens. In a study it was shown that epitope derived from TNF alpha (self antigen) when displayed on a VLP could induce antibody titer almost thousand times higher as compared to when linked to a foreign T-helper epitope (Chakerian *et al.*, 2001). Thus, the high density display of this kind is capable of breaking the B-cell energy. The magnitude of IgG induced correlated well with the density of the epitope displayed on the surface of VLP. In a recent study, VLP based vaccination strategy was tested in a PML (progressive multifocal leukoencephalopathy) patient who had idiopathic CD4⁺ T cell lymphocytopenia, a syndrome where CD4⁺ T lymphocyte count of the body is extremely low exhibiting a non-HIV (Human Immuno Deficiency Virus)- AIDS (Acquired Immuno Deficiency Syndrome)-like situation. PML is a fatal neurodegenerative brain disease caused by uncontrolled replication of JC polyomavirus (JCV) in brain of immuno-compromised individuals. The JCV VLP based vaccine could elicit humungous anti-JCV neutralizing antibody response with a parallel decline in viral load in blood and significant decrease in PML lesion size in brain (Ray *et al.*, 2015). This clearly demonstrated the efficacy of VLPs, particularly because of the fact that this patient had CD4⁺ T cell lymphocytopenia.

Quite a few if not many VLP-based vaccines have been developed successfully (Table 1) and some more are in research pipeline, at pre-clinical stage or been tested in clinical trials.

Hepatitis B vaccine (HBV vaccine) is the first recombinant protein-based vaccine for human use approved by Federal Drug Administration (FDA) in 1986. This vaccine is based on the HBV surface antigen (HBsAg)

Another highly successful VLP based prophylactic vaccine is the Human Papilloma Virus (HPV) VLP vaccine. The FDA approved HPV L1 VLP based vaccines, Gardasil (Merck) and Cervarix (GlaxoSmithKline), are extremely efficacious against cervical cancer. VLP-based vaccines available for human use are listed in Table 1.

Synthetic VLPs

Many viral surface antigens have the ability to self-assemble into VLPs that resemble the native viral particles in appearance, for example, in case of Human Papillomavirus (Kemp *et al.*, 2011; Schiller and Muller, 2015), JC polyomavirus (Ray *et al.*, 2015), BK polyomavirus (Pastrana *et al.*, 2013) etc. Many others that cannot self-assemble, or those where the viral capsid is encased inside a lipid envelope, or those where the viral surface proteins are relatively mobile/not rigid, can also potentially be displayed in a more rigid and thermodynamically stable fashion as synthetic VLPs. In such cases, either the viral antigens are displayed on another viral surface or on biomolecules like ferritin which are capable of multivalent display. An example is a nanoparticle developed for influenza, where the influenza viral hemagglutinin (HA) was fused to ferritin (Kanekiyo *et al.*, 2013). Ferritin monomers can naturally self-assemble into a nanoparticle composed of 24 ferritin monomers. Kanekiyo and colleagues could fuse Influenza HA to ferritin and found that this ferritin-HA fusion protein could self-assemble into a spherical nanoparticle displaying eight trimeric surface spikes.

In a different study, two antigen display platforms, ferritin and encapsulin were used for displaying the glycoprotein gp350 of Epstein-Barr virus (EBV), which is used by the virus for infecting B-cells (Kanekiyo *et al.*, 2015). While 24 ferritin monomers assemble to form an octahedron, encapsulin

Table 1: VLP based vaccines available for human use

Name	Target virus	Year of license	VLP size	Source
RECOMBIVAX(Merck)	Hepatitis B virus (HBV)	1986	22nm	Yeast
GARDASIL (Merck)	Human Papilloma virus (HPV)	2006	40-60nm	Yeast
CERVARIX (GlaxoSmithKline)	Human Papilloma virus (HPV)	2009	40-60nm	Baculovirus
HENCOLIN (Xiamen Innovax Biotech)	Hepatitis E virus (HEV)	2011	20-30nm	<i>Escherichia coli</i>

monomers assemble to form an icosahedron consisting of 60 subunits. Ferritin and encapsulin nanoparticles displaying EBV gp350 truncation variants could elicit high titer neutralizing antibodies in mice. After a second dose i.e. booster, there was a significant increase in neutralizing antibody titers and showed better affinity maturation. In contrast, immunization with isolated gp350 ectodomain could induce production of neutralizing antibodies, almost thousand fold less than elicited by the nanoparticle groups, thus highlighting the importance of high density display of antigens.

Very recently, Thrane and colleagues published an article where they exploited a split intein SpyTag/SpyCatcher conjugation system to develop antigen-complexed VLPs (Thrane *et al.*, 2016). This system is based on a protein domain, CnaB2 from the fibronectin adhesion protein (FbaB) of bacterium *Staphylococcus pyogenes* (Spy). CnaB2 domain locks itself together by forming a spontaneous isopeptide bond between Lys and Asp and this reaction is irreversible. Zakeri and colleagues earlier showed that CnaB2 domain could be split and engineered into two parts, a peptide (SpyTag) and a protein (SpyCatcher) and that these two could form amide bond irreversibly just by mixing the two components together (Zakeri *et al.*, 2012). VLPs expressing SpyTag or Spy Catcher can thus be linked potentially to any antigen that is

fused to either SpyCatcher or SpyTag respectively. This might inturn enable displaying otherwise difficult antigens in a much stable fashion as the bond formed is irreversible.

A Long Way to Go!

While VLPs have proven to be potential vaccine candidates in some cases, there are many hurdles to overcome so as to utilize the amazing capacity of such a vaccine tool for use against many more viral diseases. The assembly of the viral surface proteins once achieved in rigid high density thermodynamically stable conformation, should present a way to develop vaccine against many dreadful diseases; like Dengue, HIV and many more. The tool is potentially not limited only to viral diseases, but antigens from other pathogens can also be displayed on VLPs to evoke potent B and T cell responses. This highly interdisciplinary approach involving various disciplines of biology like; structural biology, computational biology, advanced electron microscopy, nanotechnology, virology, biophysics and biochemistry has gained interests of many scientists working either to understand the structure of virus, the viral entry or vaccine strategies and is been used to put forward novel, highly potent vaccine candidates to be tested in pre-clinical and clinical trials.

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