Virus-like particles as nanovaccine candidates

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Abstract
The existing vaccines are mainly limited to the microorganisms we are able to culture and produce and/or to those whose killing is mediated by humoral response (antibody mediated). It has been more difficult to develop vaccines capable of inducing a functional cellular response needed to prevent or cure chronic diseases. New strategies should be taken into account in the improvement of cell-based immune responses in order to prevent and control the infections and eventually clear the virus. Preclinical and clinical results with vaccine candidates developed as a vaccine platform based on virus-like particles (VLPs) evidenced their ability to stimulate mucosal as well as systemic immunity. Particles based on envelope, membrane or nucleocapsid microbial proteins induce a strong immune response after nasal or parenteral administration in mice, non-human primates and humans. In addition, the immune response obtained was modulated in a Th1 sense. The VLPs were also able to immunoenhance the humoral and cellular immune responses against several viral pathogens. Studies in animals and humans with nasal and systemic formulations evidenced that it is possible to induce functional immune response against HBV, HCV, HIV and dengue virus.

Keywords: VLPs, hepatitis, HBV, HCV, human immunodeficiency virus, Th1

Classification numbers: 2.05, 5.08

1. Introduction

Virus-like particles (VLPs) are inert nanoparticles which contain no DNA/RNA from the virus itself. However, VLPs may reproduce the structure and size of the virus particle and can be engineered to have inserted, coupled or aggregated homologous or heterologous antigens. By extension, particles that contain antigens from non-viral sources and show similar size and shape as viruses are also regarded as VLPs. VLPs-displayed antigens are efficiently taken up by professional antigen presenting cells (APCs) and induce potent immune responses after parenteral or mucosal immunizations [1–3].

Several strategies have been used to produce a given antigen with capacity to form VLPs, or to obtain it as part of a recombinant protein forming VLPs. Antigens repeated on VLPs, like those naturally found in viral capsids, efficiently cross-link B-cell receptors and, therefore, induce strong IgG responses. VLPs can be used as a carrier of heterologous antigens to enhance the immunological response against poorly immunogenic regions [4].

One of the most relevant examples of VLPs in the history of vaccinology has been the recombinant hepatitis B surface antigen (HBsAg), produced as VLPs in Saccharomyces cerevisiae, Pichia pastoris or other yeasts or mammalian cell hosts. It has been used for more than 20 years as a very effective vaccine in the prevention of hepatitis B. In 2006, the US Food and Drug Administration (FDA) approved a human papilloma virus (HPV) vaccine for clinical use, that consists of HPV 6, 11, 16 and 18 recombinant VLPs mixed with
Figure 1. Mean particle size for NASVAC batches as measured by DLS. Batches produced from 2005 to 2010 evidenced a consistently similar size of aggregated VLPs from 50 to 60 nm mean size.

Figure 2. Physical aspect of HBcAg (a), HBsAg (b) and the combined formulation (c). It is easy to note the ability of both antigens to aggregate in their phosphate saline buffer and pH conditions.

The immunological properties of NASVAC may be explained by the physical and temporal association of HBsAg and HBcAg in aggregates of nanometer size. Such aggregates of VLPs are stable structures of 50–60 nm according to the evaluation of several batches produced in a period of 6 years (figure 1). The presence of such aggregates may justify the cross-enhancement of the immunity against both antigens.

The concept of using mucosal immunization is a novel concept in therapeutic vaccination. Moreover, in the field of hepatitis B therapeutic vaccine development, this is the first
candidate to include the hepatitis B nucleocapsid antigen as part of the formulation. Clinical trials in chronically infected patients are currently ongoing.

3. Virus-like particle technology in human immunodeficiency virus (HIV) vaccine development

Cell-mediated immune response to HIV-1 is an essential component of the mechanisms toward the viral replication control. In this regard potent Th1 adjuvants are required to develop novel vaccine candidates. On the basis of previous evidence obtained in the NASVAC project it was hypothesized that the mixture of the VLPs HBsAg and HBCAg would act as a Th1 adjuvant for co-administered antigens. This led to the application of the concept to the HIV research, where a soluble, multiepitopic antigen (named CR3) was developed that included several Th and CTL rich regions from HIV-1 proteins [11].

According to our studies in mice, the multiantigenic formulation TERAVAC-HIV-1 (figure 3) comprising CR3 with nanoparticles of HBV induces anti-CR3 cellular responses after nasal and parenteral inoculations and best results are obtained in schedules with simultaneous parenteral and nasal co-administration [12, 13]. We have found a strong Th1 bias of the CR3-specific response, the induction of CD4+ and CD8+ T cells in mice’s spleen and IFN-γ-secreting cells in mesenteric lymph nodes. The induction of CD8+ cells might be explained because of the non-covalent interaction of CR3 with the VLPs [14], which enables the crosspriming after entry and processing of the recombinant protein inside APC following the same routes as the HBV VLPs. Additionally, we also detected anti-HBsAg and anti-HBcAg cellular and humoral responses. In this regard, our multiantigenic formulation might provide immunity to HBV as well, which would be of additional benefit considering the high HIV–HBV coinfection rate reported worldwide.

Preclinical evaluation of this multiantigenic vaccine candidate was conducted up to the demonstration of the safety and immunogenicity of the formulation and a phase I therapeutic clinical trial is ongoing.

In a second set of experiments it was demonstrated that the coupling of multiantigenic peptides (MAPs) to the HBsAg VLP greatly improved the immunogenicity and the crossreactivity of the humoral immune response, opening a new window of practical use to such VLP structures as adjuvants in the field of HIV vaccines [14].

4. VLPs of hepatitis C virus core protein as components of vaccine candidates against HCV

Hepatitis C virus (HCV) infection is a worldwide health problem and no vaccine is available yet. Virus-like nucleoparticles (VLNs), based on HCV core protein, are currently being evaluated as components of vaccine candidates against this pathogen. A variant comprising the first 120 aa of HCV core antigen (HCCAg.120) has been obtained from recombinant Escherichia coli strain [15]. This protein variant self-assembles in particles of approximately 15 nm (figure 4). These HCCAg.120-VLNs have demonstrated to be immunogenic in animal models [15, 16]. Particularly, HCCAg.120-VLNs induced high titers of anti-HCCAg.120 antibodies and elicited specific delayed type hypersensitivity, as well as generating a predominant Th1 cytokine profile in immunized mice [16].

Immunological parameters correlating with protection of HCV infection have not been established. However, induction of neutralizing antibodies as well as cell-mediated immune response against several viral antigens is expected to be required for protection against HCV chronic infection [17]. Therefore, vaccine candidates must be able to induce both humoral and cellular immune responses and target several viral antigens. Taking this into account, HCCAg.120-VLNs have been evaluated as components of vaccine candidates in combination with other molecules.

In the vaccine candidate CIGB-230, HCCAg.120-VLNs are mixed with pIDKE2 [18], a plasmid for DNA
immunization expressing HCV core, E1 and E2. This plasmid has previously shown its ability for eliciting specific immune response against the capsid and envelope proteins in rabbits and Macaca irus [18]. Interestingly, in this preparation the interaction between protein and DNA components was demonstrated and HCCAg.120-VLNs were larger than those observed in the absence of DNA [19]. The CIGB-230 vaccine candidate has demonstrated to be safe and immunogenic in animal models and in HCV-chronically infected humans, with the induction of neutralizing antibodies and cellular immune response [17]. In another approach, HCCAg.120-VLNs have been mixed with protein variants of HCV envelope antigens [20]. This preparation has demonstrated to be immunogenic in mice and African green monkeys, eliciting humoral and cellular responses against all HCV structural antigens and protecting in a surrogate challenge model [21].

The clinical impact of these strategies has not been demonstrated yet. However, the above-mentioned elements indicate that the use of VLNs as components of vaccine candidates against HCV is a promising approach for the development of rational and effective interventions against this pathogen.

5. Vaccine candidate against dengue based in a VLP of the capsid protein

The Cuban approach relies on the subunit vaccine against dengue based on two different viral regions: the domain III of the Envelope protein and the capsid protein. Domain III of the E protein has been proposed for involvement in receptor recognition [22], as supported by several studies [23–25]. We have also described the functionality of fusion proteins containing domain III of the E protein from dengue virus (DENV) in terms of their induction of neutralizing antibodies and protection in mice and monkeys [26–30]. Therefore, this region, properly folded and presented to the immune system, is an inducer of neutralizing antibodies against dengue.

In parallel, due to the fact that cell-mediated immunity (CMI) has also been recently recognized as an important factor in protection against DENV in mice, we obtained nucleocapsid-like particles (NLPs) with around 25 nm of diameter, from the recombinant dengue-2 capsid protein (C-2), expressed in E. coli. Upon mice immunizations, animals induced a serotype-specific protection in the mouse encephalitis model. Furthermore, this protection was mediated by CD4+ and CD8+ cells without the induction of a functional humoral immune response against DENV [31, 32].

In addition, we also designed and obtained a novel chimeric protein from DENV-2: domain III-capsid (DIIC-2), which contains viral fragments and is potentially an inducer of neutralizing antibodies and CMI. This molecule was efficiently produced in E. coli, and it could be properly folded and purified. When it was presented as a particulate aggregate including at random oligodeoxynucleotides, it induced antiviral and neutralizing antibodies, CMI, and conferred a significant level of protection in mice [33]. Recent results also revealed the profile of serotype-specificity associated to both arms of the immune response generated in mice with this chimeric protein [34].

Finally, particles based on the DIIC-2 protein were assessed in dengue-positive non-human primates. Upon protein administration, animals exhibited a significant boost effect in terms of neutralizing antibodies and CMI [35]. Taken together, we can assert that nanoparticles of the chimeric protein DIIC-2 protein are highly immunogenic in mice and monkeys, being suitable for a future vaccine candidate against dengue.

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