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Virus-Like Particles as vaccine antigens and adjuvants: application to chronic disease, cancer immunotherapy and infectious disease preventive strategies

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Abstract

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 responses. It has been more difficult to develop vaccines capable to induce functional cellular responses needed to prevent or cure chronic diseases. Several results suggest that specific enhancement of T-cell responses is nevertheless possible in persistently infected patients to treat chronic diseases including cancer. This work presents preclinical and clinical results obtained using virus like particles (VLPs) as a vaccine platform. VLPs based on envelope, membrane or nucleocapsid microbial proteins are able to stimulate mucosal as well as systemic immunity and induce a strong immune response after nasal or systemic administration in mice, non human primates and humans. In addition, the immune response obtained is biased in a Th1 direction. VLPs were able to potentiate humoral and cellular immune responses against several viral and cancer antigens as measured by LPA and IFN-γ ELISPOT assays. Studies in animals and humans with nasal and systemic formulations show that it is possible to induce functional immune responses against HBV, HCV, dengue as well as against prostate and cervical cancers.

Keywords: *Virus-like particles; Adjuvant; Antigen; Vaccine; Cellular immunity; Humoral immunity*

1. Introduction

Virus-like particles (VLPs) are inert, empty viral capsids, which contain no DNA/RNA from the virus itself. However, they retain the structure of a virus particle and can be engineered to have antigens attached. By extension, particles that contain antigens from viral or non-viral sources and show similar size and shape as viruses are also regarded as VLPs. VLPs-displayed antigens are efficiently taken up by dendritic cells (DC) and induce potent immune responses after parenteral, mucosal or transcutaneous immunization [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. Recent studies have shown the

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improvement in the immunogenicity of covalently coupled peptides from the allergen Der p 1 to a VLP derived from bacteriophage Qbeta (Qbeta-Der p 1), demonstrating that this strategy can be used to enhance the efficiency of allergen-specific immunotherapy and validate the approach in humans [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* or *Pichia pastoris* yeasts. It has been used for more than 20 years as a very effective vaccine in the prevention of Hepatitis B. More recently, in 2006, the U.S. 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 an aluminum-containing adjuvant. This was a very positive advancement in the use of VLPs for the development of new vaccines.

2. Virus-like particle technology in chronic hepatitis B immunotherapy

The entire recombinant core antigen (HBcAg) of the hepatitis B virus (HBV) promotes Th1 immunomodulation of the immune response to coadministered antigens, including the HBV surface antigen (HBsAg), after subcutaneous [5] or nasal [6] inoculations. Riedl and coworkers have related this adjuvant activity to the nucleic acid content in the particle, which interacts with Toll-like receptor-3 (TLR3) [7]. In addition, we have observed a synergistic effect in the enhancement of the immunogenicity of both the surface and core antigens after nasal administration, indicating that HBcAg increases the anti-HBsAg response and that, conversely, HBsAg increases the specific anti-HBcAg response [8].

These results led in 1998 to formulate a new VLP-based nasal therapeutic vaccine candidate for chronic hepatitis B therapy under the name NASVAC, that contained the surface and core HBV antigens (see **Figure 1** below). The candidate vaccine induced stronger cellular and humoral (IgG2a) immune responses than a commercial HBsAg vaccine in mice. These results were in favour of the therapeutic use of the vaccine, as Th1-biased immune responses correlate with control of HBV [9, 10]. A double blinded, randomized and placebo-controlled Phase I clinical trial was therefore launched in healthy volunteers, evidencing safety and immunogenicity of this nasal vaccine candidate with a relatively low amount of antigen administered (50 μg each) [11]. This is the first nasal candidate vaccine developed for hepatitis B therapy, taking advantage of the cross-adjuvanting effect of the VLPs included in the formulation [5-8].

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 whose results are expected to be published by the end of 2010.

3. Virus-like particle technology in Human Immunodeficiency Virus immunotherapy

Cell-mediated immune responses to HIV-1are an essential component of 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 a mixture of the HBsAg and HBcAg VLPs would act as a Th1 adjuvant. This led to the application of the concept to HIV research, where a soluble, multiepitopic antigen (named CR3) was developed that includes several Th and CTL epitopes from HIV-1antigens [12].

In mice, the multiantigenic formulation comprising CR3, HBcAg and HBsAg induced detectable anti-CR3 cellular responses after nasal and parenteral inoculations. Best results were obtained in schedules that combined parenteral and nasal co-administrations [13,14]. A strong T helper cell (Th)-1 bias of the CR3-specific response was observed, together with the induction of $CD4^+$ and $CD8^+$ T cells in mice spleens and IFN-γ-secreting cells in mesenteric lymph nodes. 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. Preliminary studies suggest that the adjuvant effect of HBV antigens on the CR3-specific immune response depends on the physical interaction among the proteins [13] and requires co-inoculation of the antigens [15]. Preclinical evaluation of this multiantigenic vaccine

candidate was conducted up to the demonstration of the safety and immunogenicity of the formulation. A clinical Phase I trial is planned to begin in 2010.

In a second set of experiments, it was demonstrated that the coupling of multiantigenic peptides (MAPs) to HBsAg VLPs greatly improved the amplitude and crossreactivity of the resulting humoral immune response, opening a new window for the practical use of VLP structures as adjuvants in the field of HIV vaccines [16-18].

4. Very small size proteoliposome (VSSP) technology applied to cancer therapy

A novel structure used as an adjuvant is a nanoparticulate hydrophobic conjugate in which purified gangliosides were incorporated into the outer membrane protein complex of *Neisseria meningitidis* to form Very Small Sized Proteoliposomes (VSSP), a type of small (9 nm) vesicles with strong adjuvanticity and antigen delivery properties [19]. Among the most remarkable properties of VSSP is their capacity to activate dendritic cells through the display of powerful danger signals, resulting in the enhancement of CTL and antibody responses to coadministered or inserted peptides and proteins. VSSPs also show strong capacity to prime $CD8^+$ T cell responses without a need for $CD4^+$ T cell help and offer the possibility to be used as an antigen delivery system [20].

VSSP adjuvant technology has been applied to the development of cancer therapy projects, specifically in the fields of therapeutic vaccines against Human Papillomavirus (HPV) and prostate cancer, using the antigen delivery system ability of VSSP to display heterologous peptides from HPV or GnRH, respectively [21, 22].

The formulation containing VSSP and the GnRH m1-TT peptide adjuvanted in Montanide ISA-51 was injected to prostate cancer patients in a Phase I clinical trial. It took four bi-weekly injections followed by three monthly injections to detect significant levels of antibodies to GnRH in all vaccinated patients, in spite of the immune tolerance to this self antigen. PSA and testosterone levels decreased to very low levels in the patients, even lower than castration levels (1.75 nM/L), and remained at such basal levels during a 25 months follow-up. Clinically, all patients remained with a normal prostate size and were free of serious urinary manifestations [22].

A therapeutic vaccine candidate against HPV using the VSSP adjuvant technology was evaluated in a mice tumor model using a CTL epitope of HPV [21]. A Phase I study was launched to apply this novel vaccine candidate to HPV-infected women with high degree cervical lesions. An important percentage of the patients evidenced virus clearance as determined by PCR and more than 50% of the lesions went into remission, suggesting that the treatment was both safe and effective [24].

5. Virus-like particle technology applied to dengue virus prevention

VLPs from the recombinant dengue-2 capsid protein were obtained by submitting the recombinant protein produced in *E. coli* to an *in vitro* assembly reaction using single-stranded DNA. As a result, particles of around 30 nm were obtained independent of the specificity and the length of the oligonucleotide used (**Figure 1**) [24]. These VLPs were adjuvanted with alum and inoculated to mice. The animals did not show evidence for antiviral antibodies, but their splenocytes secreted high levels of IFNγ upon virus stimulation, and a significant protection rate was achieved after lethal challenge with dengue-2 virus. Both IFNγ secretion and protection against viral challenge were dependent on $CD4^+$ and $CD8^+$ T cells [25].

In parallel, we also constructed a novel chimeric protein comprising the Domain III region of the envelope protein and the capsid protein from dengue-2 virus, in the hope to obtain a molecule potentially able to induce both humoral and cell-mediated immunity. The fusion protein was mixed with oligodeoxynucleotides to obtain particulate aggregates and tested in mice in comparison with non-aggregated control preparations.

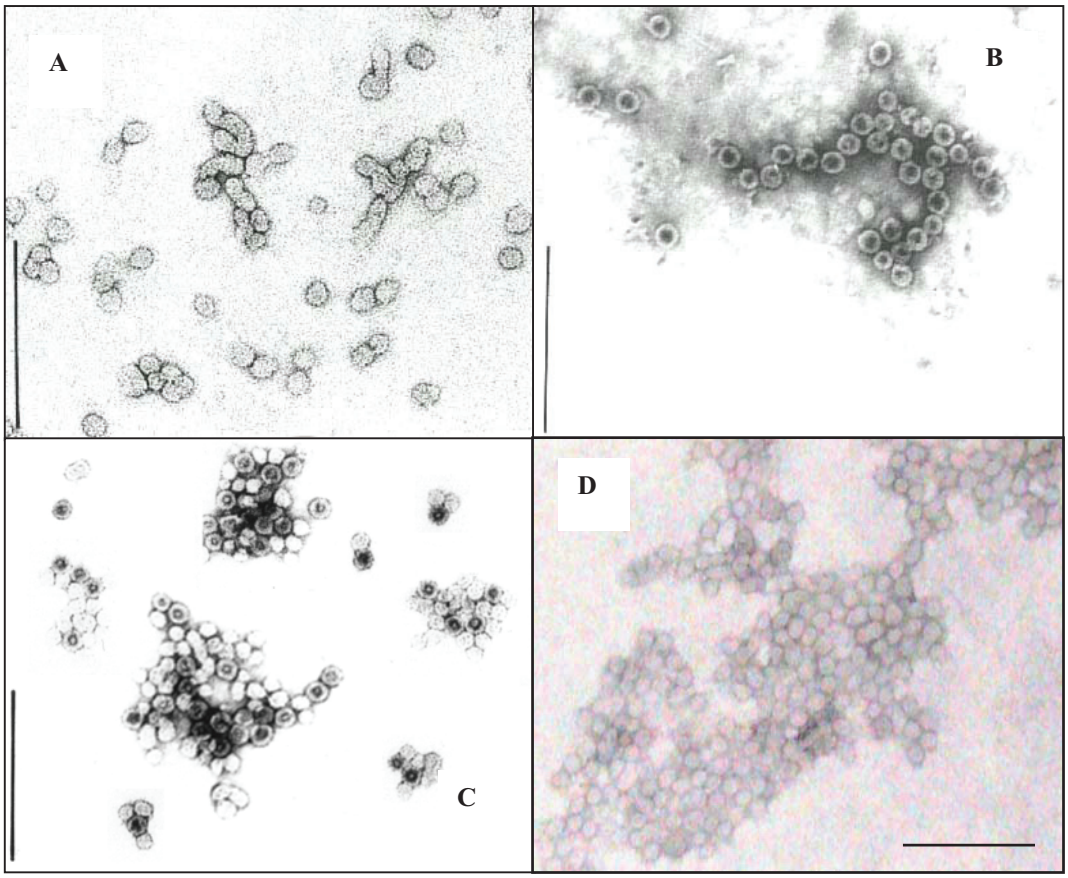

Figure 1. Hepatitis B Virus virus-like particles a) HBsAg; b) HBcAg; c) NASVAC: formulation containing a mixture of HBsAg and HBcAg (1:1); d) Dengue virus VLPs comprising the nucleocapsid antigen.

Although a similar humoral immune response was induced by both forms of the protein, the aggregated particles elicited a much stronger cellular immune response mediated by $CD4^+$ and $CD8^+$ T cells, as measured by *in vitro* IFNγ secretion and by protection experiments [26]. When the two kinds of particulate antigens were mixed with an additional fusion protein containing epitopes from the envelope glycoprotein of dengue-4 virus, an increase in the protective immune response was obtained upon DEN-4 challenge of the immunized mice. All these results demonstrate the suitability of VLPs to induce a proper immune response against dengue virus.

6. Virus-like particle technology applied to chronic hepatitis C immunotherapy

The VLP technology is being used also in the development of a vaccine against hepatitis C virus. CIGB-230 is a vaccine candidate based on the mixture of a recombinant truncated HCV core protein, which is able to self-assemble into VLPs (27), and a plasmid for DNA immunization, pIDKE2, that expresses the HCV structural antigens (Core, E1, E2) of a HCV genotype 1b isolate [28]. The plasmid component induces both humoral and cellular immune responses against Core, E1 and E2, while the recombinant core protein mainly elicits antigen-specific $CD4^+$ T cell responses [29, 30].

After satisfactory pre-clinical studies, where CIGB-230 showed low toxicity in rodents [31], a Phase I clinical trial was carried out with fifteen HCV genotype 1b-infected patients who had remained non-responders to previous IFN + Ribavirin therapy. The vaccine candidate was well tolerated and immunogenic in these difficult-to-treat HCV-

chronically infected individuals [32]. Neutralizing antibody response against heterologous HCV pseudoparticles was modified in eight individuals, including six *de novo* responders. In addition, a significant increase in the number of individuals having detectable lymphoproliferative responses and IFN-γ secretory responses against HCV structural antigens was observed during treatment. Most importantly, 46.7% of the individuals responded positively against more than one antigen present in the vaccine candidate, and more than 40% of the vaccinated individuals improved or stabilized their liver histology, particularly reducing fibrosis, which correlated with the *de novo* induction of a cellular immune response against more than one HCV antigen [33].

The enhanced functional immune response observed after administration of the vaccine candidate CIGB-230 may be due to a synergistic action between its two components, plasmid and protein, perhaps due to the reduced degradation of plasmid DNA and to a better activation and antigen presentation by antigen presenting cells [34]. The CIGB-230 vaccine candidate is currently in Phase II clinical trial in HCV-chronically infected individuals.

7. Conclusions

VLP technology has been widely explored at the Center for Genetic Engineering and Biotechnology, Cuba, evidencing the potentialities of such particles to promote immune responses due to their unique capacity to activate innate and adaptive immune responses. VLPs elicit a potent immune response against themselves and against homologous or heterologous antigens, which are coadministered with or inserted into the VLPs. VLPs can be exploited as a platform to increase the immunogenicity of poorly immunogenic antigens, including self proteins.

All these features support the possible use of VLPs expressed in *Pichia pastoris* or *E. coli* as therapeutic and/or prophylactic vaccine candidates to deal with the immunotherapy of chronic diseases and cancer as well as to be applied to preventive settings in “difficult” sceneries as HCV, HIV and Dengue virus vaccine development.

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