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Co-administration of non-carrier nanoparticles boosts antigen immune response without requiring protein conjugation

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ABSTRACT

Nanotechnology promises a revolution in medicine including through new vaccine approaches. The use of nanoparticles in vaccination has, to date, focused on attaching antigen directly to or within nanoparticle structures to enhance antigen uptake by immune cells. Here we question whether antigen incorporation with the nanoparticle is actually necessary to boost vaccine effectiveness. We show that the immunogenicity of a sub-unit protein antigen was significantly boosted by formulation with silica nanoparticles even without specific conjugation of antigen to the nanoparticle. We further show that this effect was observed only for virus-sized nanoparticles (50 nm) but not for larger (1000 nm) particles, demonstrating a pronounced effect of nanoparticle size. This non-attachment approach has potential to radically simplify the development and application of nanoparticle-based formulations, leading to safer and simpler nanoparticle applications in vaccine development.

1. Introduction

Nanotechnology is opening new horizons in vaccinology. This technological innovation, termed nanovaccinology [1], uses nanoparticles to adjuvant vaccine formulations. Soluble protein antigens are attached to nanoparticles to particulate them and enhance uptake by antigen presenting cells (APCs) [2]. The uptake and processing of antigens by cells depends on how the proteins are presented to the immune system. Physical factors including size, shape, surface charge, hydrophobicity and hydrophilicity, as well as receptor interactions, all influence the outcome [3–6]. As cells effectively process antigens with dimensions similar to those of pathogens such as viruses (20–200 nm) and bacteria (0.5–5 μm) [7], much effort has been directed at particulating soluble antigens by attaching them to nanoparticles having dimension in these ranges.

Based on this well-accepted "must-be-attached" paradigm, the design of nanoparticle vaccines inherently involves engineering or surface manipulation to promote association of antigen and nanoparticle, such as by chemical conjugation, encapsulation, or adsorption [2]. Most attachments are achieved through multiple steps of chemical reaction or physical stress that can add significant complexity and cost to the entire process, and may structurally alter the antigen or increase formulation toxicity [8,9]. Indeed, association could be detrimental considering the well-known labile nature of conformational proteins, especially when placed near surfaces. Although studies have demonstrated high immunogenicity of target antigens adsorbed, entrapped or conjugated with poly(lactide-co-glycolide) [10], *N*-trimethyl chitosan [11], polymethylmethacrylate [12], or calcium phosphate [13], direct comparisons of the same antigen and nanoparticle formulations, with and without conjugation, are lacking. Indeed, such comparisons may not be feasible, as identical chemistries having different states of attachment are, by definition, impossible to achieve. This nevertheless leads us to ask whether association of the nanoparticle and antigen is always necessary, particularly for more complex antigens which already have viral architecture.

In this study, we question whether the prevailing approach of antigen attachment to a "carrier" adjuvant is necessary. We approach this question using a sub-unit viral protein capsomere, which already has a viral molecular signature. A capsomere is the basic building block of a virus-like particle, a highly ordered assembly of viral structural proteins (Fig. 1). Recently, a modular capsomere was designed through a process of synthetic biology to give a sub-unit vaccine design which combines the advantages of

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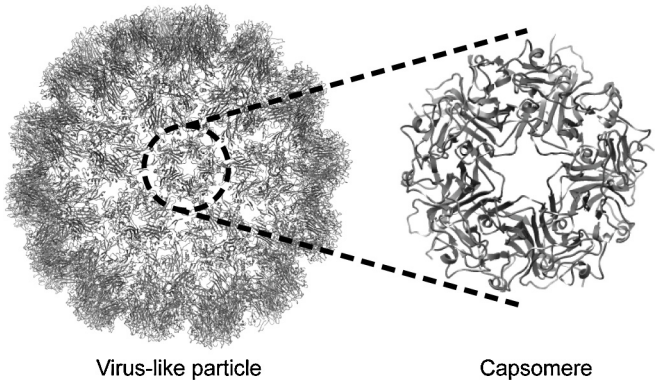

Fig. 1. Murine polyomavirus-like particle (1sid.pdb); each particle comprises 72 capsomeres.

repetitive antigen presentation on a virus-like particle (VLP) subunit with the efficiency of microbial manufacturability [14,15]. This capsomere has been reported to induce high antibody titers which were boosted more than 10-fold by formulation with conventional aluminum-based adjuvant, confirming the critical role adjuvants play [16,17].

It is conceivable that stimulating the immune system with a viral molecular signature, provided by the capsomere, and also a nanoparticle of virus size, is sufficient to increase antigen immunogenicity. If so, the need for attachment of the protein to the nanoparticle is circumvented. A simple mixing approach to vaccine formulation (Fig. 2) is then enabled. Such an approach is often used for soft-matter adjuvants such as those based on emulsion (AS03^{TM} and MF59^{TM}), saponin (ISCOMATRIX^{TM}) or inulin (Advax^{TM}), although these have quite different chemistry to solid nanoparticles [18–21].

Here, we show that the immunogenicity of a capsomere protein antigen was significantly boosted by formulation with silica nanoparticles even though there was no significant attachment of the antigen to the nanoparticle. We further discovered that this effect was observed only for virus-sized nanoparticles (50 nm) but not for larger (1000 nm) particles. These results suggest that, for non-aggregated virus-sized nanoparticles, it may not be necessary to attach antigen to the nanoparticle, and that the simple presence of these nanoparticles in a vaccine formulation may boost immune response.

2. Materials and methods

2.1. Plasmid construction

Expression vector for CapM2e (previously named construct 1011) was as described previously [15].

2.2. Protein expression and purification

Expression vector for CapM2e was transformed into chemically competent *E. coli* Rosetta (DE3) pLysS cells (Novagen, CA, USA). GST-tagged CapM2e was expressed and purified to yield low-endotoxin CapM2e capsomeres (<2 EU mg^{-1} protein) as previously described [15,22,23]. Capsomeres concentration was adjusted to 2 mg mL^{-1} with PBS. Endotoxin level was analyzed using LAL-based assay Endosafe PTS^{TM}-2005 (Charles River Laboratory, MA, USA).

2.3. Transmission electron microscopy

Commercial silica nanoparticles of nominal diameter 50 nm and 1 μm (Cat. 24040 and 24326, Polysciences Inc., USA) were dialyzed against PBS at 4 °C for 24 h. Nanoparticles were analyzed by transmission electron microscopy. Briefly, 2 μL of silica nanoparticles at a nominal silica concentration of 1 mg mL^{-1} was applied to glow-discharged, 200-mesh carbon-coated grids (Proscitech, QLD, Australia). Remaining liquid on the grids was blotted with filter paper after 2 min. Grids were washed with water, dried and

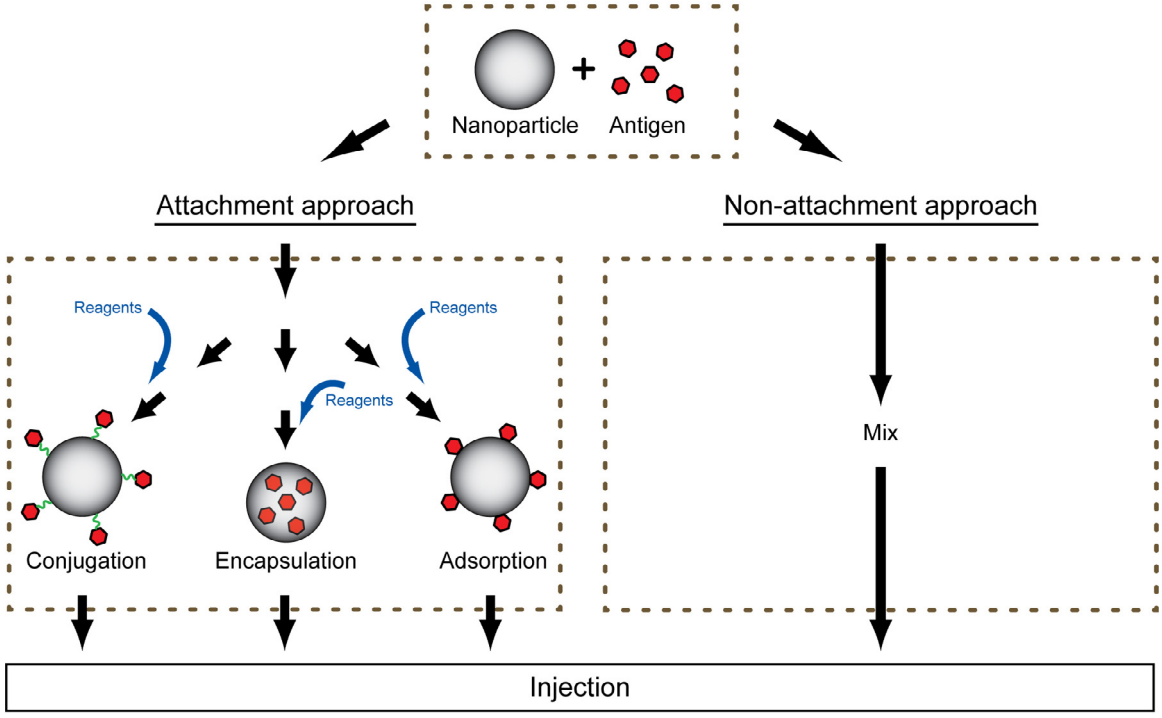

Fig. 2. Attachment and non-attachment approaches for antigen–nanoparticle formulation. Attachment involves complex multiple steps. In contrast, the non-attachment approach involves only single-step simple mixing of antigen and nanoparticle shortly prior to injection.

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