NANOVACCINES AGAINST SARS-COV-2: HOW THE INCORPORATION OF DIFFERENT CATEGORIES OF NANOPARTICLES TO A SUBUNIT VACCINE AFFECTS THE QUALITY MORE THAN THE QUANTITY OF INDUCED ANTIBODIES

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Abstract:
The development of anti- SARS-CoV-2 vaccination strategies remains a central issue worldwide even after the commercial approval of several formulations. From all strategies being studied, protein-based formulations could be a promising strategy specially for being safer and not using full pathogens. However, protein-based subunit vaccines are generally less immunogenic, which could be counteracted by using adjuvants and, most of all, nanoparticles. When it comes to nanovaccines, different classes of nanoparticles can be used and they may interact with the immune system in different ways, being subject to a plethora of factors that affect its efficiency. Here we propose the use of two different nanovaccine strategies aiming to boost the antibody response against the Spike protein receptor binding domain (RBD), the most relevant SARS-CoV-2 antigen target. First, we used gold nanoparticles (AuNP) to which the antigen is adsorbed. The AuNPs were developed in 3 different sizes according to reports that NP size may contribute to the biodistribution *in vivo* and may determine the interaction with the immune system. Second, we employed lipid nano multilamellar vesicles (NMVs) that incorporate the antigen in its structure together with the FDA-approved lipid adjuvant, the monophosphoryl lipid A (MPLA), aiming to stablish the contribution of each element of the composition. To assess the potential of the different classes of nanoparticles to boost humoral responses in a murine model, C57BL/6 mice were immunized with 2 or 3 doses of RBD alone or incorporated in the different nanovaccine formulations. For the AuNP-based nanovaccines, we observed that the presence of the nanoparticle in the formulation has only a subtle effect in the magnitude of the antigen-specific serum IgG response. However, measurement of neutralizing antibodies reveals that the presence of AuNP in the formulation had a positive effect and boosted the induction of antibodies that effectively block viral infection *in vitro*. The size of AuNPs seems to play an important role, but even though larger particles were more effective at inducing neutralizing antibodies, this phenomenon is observed in response to all AuNP-based nanovaccines here assessed. For the NMV-based formulations, we also observed that the presence of the nanoparticle in the formulation had no extraordinary effect on the overall antigen-specific IgG titers, but instead strongly contributed to the induction of neutralizing antibodies that capable of blocking SARS-CoV-2 *in vitro*. For this formulation specifically, the synergistic interaction of NMV and MPLA allowed the use of one less dose (2-dose regimen instead of 3) to reach similar performance. Overall, our results clearly indicate that the incorporation of nanoparticles in vaccine formulations has a more pronounced effect on the quality and functionality of the antibodies induced than in the quantity, allowing us to demonstrate that nanovaccines may represent the bridge that leads subunit vaccines to the next level of efficiency required for an expansion in the possible applications of this class of vaccines.
keywords: nanovaccines; SARS-CoV-2; nanoparticles; neutralizing antibodies;

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