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Research Highlights on a Special Focus issue on Vaccine Development using nanoparticles

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Designer aluminum salt-based nanoparticles as vaccine adjuvants

Evaluation of: Bingbing Sun, Zhaoxia Ji, Yu-Pei Liao *et al.* Engineering an Effective Immune Adjuvant by Designed Control of Shape and Crystallinity of Aluminum Oxyhydroxide Nanoparticles. *ACS Nano*. 7(12), 10834–10849 (2013).

Aluminum salts, such as aluminum hydroxide, are widely used in vaccines to improve antigen-specific immune responses (1). Despite their widespread usage, the exact mechanism of their action is still not yet fully elucidated. Recently, NLRP3 inflammasome, also known as NALP3 or cryopyrin, has been implicated in the immunostimulatory effects observed with aluminum salts (2). Structural studies using X-ray diffraction and infrared spectroscopy have shown that aluminum hydroxide adjuvant is actually crystalline aluminum oxyhydroxide (AlOOH), as opposed to Al(OH)₃. The geometrical shape of the adjuvant in general has also been implicated in its uptake by antigen-presenting cells (APC). In this study, Sun *et al.* designed aluminum oxyhydroxide nanoparticles with different morphologies, shapes and hydroxide concentrations and investigated their adjuvant activities. They studied the effect of these parameters on aluminum oxyhydroxide's ability to trigger the NLRP3 inflammasome and APC uptake, relative to a commercial alum preparation, using *in vitro* assays and *in vivo* studies. An AlOOH library of nanorods, nanoplates and nanopolyhedral were synthesized using a hydrothermal method and structures confirmed by TEM, XRD, TGA and FTIR analyses. All the nanostructures were able to activate the NLRP3 inflammasome through generation of oxidative stress in THP-1 human monocytes and induce maturation of mouse bone marrow derived dendritic cells and IL-1 β cytokine production by them, but the nanorods were more effective than the nanoplates, nanopolyhedra and the commercial alum. The nanorods were hence used in an *in vivo* immunization study using ovalbumin (OVA) as a model antigen. The OVA-specific immune responses induced by the OVA-adsorbed aluminum oxyhydroxide nanorods tended to be stronger than that induced OVA-adsorbed on the commercial alum preparation. This study opens the possibility of engineering designer aluminum salt-based adjuvants with a stronger adjuvant activity.

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Size does matter in aluminum salt-based vaccine adjuvants

Evaluation of: Xinran Li, Abdulaziz M. Aldayel and Cui Zhengrong. Aluminum hydroxide nanoparticles show a stronger vaccine adjuvant activity than traditional aluminum hydroxide microparticles. *Journal of Controlled Release* 173, 148–157 (2014).

Aluminum salts, including aluminum hydroxide, aluminum phosphate, and aluminum potassium sulfate, are commonly used in human vaccines for over 70 years (2). Despite their demonstrated favorable safety profile, aluminum salts can only weakly or moderately enhance the immunogenicity of antigens adsorbed on them. The primary particles of aluminum salts are in the nanometer-scale. For example, the primary fibers in aluminum hydroxide crystalline powder have an average dimension of $4.5 \times 2.2 \times 10$ nm (3). However, when they are dispersed in an aqueous solution, the primary particles aggregate to form larger microparticles of 1–20 μ m (3). Because it is well known that the size of particulate adjuvants significantly affect their vaccine adjuvant activity (4), Li *et al.* synthesized $\text{Al}(\text{OH})_3$ nanoparticles of ~110 nm in diameter and compared their adjuvant activity with larger $\text{Al}(\text{OH})_3$ microparticles of ~9.3 μ m in diameter using OVA and *Bacillus anthracis* protective antigen (PA) protein as model antigens. They showed that antigens adsorbed on the aluminum hydroxide nanoparticles induced stronger and more durable specific antibody responses than the same antigens adsorbed on aluminum hydroxide microparticles. The aluminum hydroxide nanoparticles were more effective than the microparticles in facilitating the internalization of antigens adsorbed on them by APCs. Moreover, the local inflammatory response induced by the nanoparticles appeared to be weaker than those induced by microparticles. For years, scientists have been searching for new materials that can be used as vaccine adjuvants, with very limited success. The research papers by Li *et al.* (2014) and Sun *et al.* (2013) clearly pointed out that an alternative and potentially more feasible strategy to find better vaccine adjuvants may be to innovatively modify the physical properties of aluminum salts.

To be inside or outside that is the question

Evaluation of: Weifeng Zhang, Lianyan Wang, Yuan Liu *et al.* Immune responses to vaccines involving a combined antigen-nanoparticle mixture and nanoparticle-encapsulated antigen formulation. *Biomaterials* 35, 6086–6097 (2014).

Nano- and micro-particles prepared using the biodegradable and biocompatible polymer poly (lactic-co-glycolic acid) (PLGA) have been extensively studied and used for vaccine and drug delivery. One important aspect of vaccine formulation has been the antigen release kinetics, leading to antigen persistence and prolonged release (2). Methods used to load antigens to nanoparticles have been entrapment (or encapsulation), surface physical adsorption, and surface chemical conjugation (4). Zhang *et al.* designed a study to investigate the optimal method to load antigens to PLGA nanoparticles using OVA as a model antigen. They loaded OVA to PLGA nanoparticles in three different ways, namely encapsulation, physical mixture, or a combination of encapsulation and physical mixture. Encapsulation offers the best protection of the antigen, but the release of the antigen from the nanoparticles was slow. Physical mixture resulted in a faster antigen release instead

(even in the physical mixture, there likely were some interactions such as H-bonds, van der Waal forces, electrostatic interactions, between the nanoparticles and the antigens). It was found that the combined formulation of encapsulation and physical mixture of antigens gave qualitatively and quantitatively superior immune responses relative to encapsulation and mixture alone. The authors attribute the superior responses to the combined formulation, which likely provided an adequate initial antigen delivery from the mixture formulation followed by a slow release of antigens from the encapsulated formulation. This work provides useful insights into formulating vaccines with superior immunogenicity by fine tuning the release kinetics of the antigen from the vaccine formulations.

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