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NANOMEDICINE

Strong antibody responses induced by protein antigens conjugated onto the surface of lecithin-based nanoparticles

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ABSTRACT

An accumulation of research over the years has demonstrated the utility of nanoparticles as antigen carriers with adjuvant activity. Herein we defined the adjuvanticity of a novel lecithin-based nanoparticle engineered from emulsions. The nanoparticles were spheres of around 200 nm. Model protein antigens, bovine serum albumin (BSA) or *Bacillus anthracis* protective antigen (PA) protein, were covalently conjugated onto the nanoparticles. Mice immunized with the BSA-conjugated nanoparticles developed strong anti-BSA antibody responses comparable to that induced by BSA adjuvanted with incomplete Freund's adjuvant and 6.5-fold stronger than that induced by BSA adsorbed onto aluminum hydroxide. Immunization of mice with the PA-conjugated nanoparticles elicited a quick, strong, and durable anti-PA antibody response that afforded protection of the mice against a lethal dose of anthrax lethal toxin challenge. The potent adjuvanticity of the nanoparticles was likely due to their ability to move the antigens into local draining lymph nodes, to enhance the uptake of the antigens by antigen-presenting cells (APCs), and to activate APCs. This novel nanoparticle system has the potential to serve as a universal protein-based vaccine carrier capable of inducing strong immune responses.

1. Introduction

Recombinant protein antigens may help overcome the toxicity concerns associated with many of the traditional vaccines prepared with live, attenuated or killed pathogens. However, recombinant proteins are often weakly immunogenic or non-immunogenic on their own, and a vaccine adjuvant is usually needed to enhance the resultant immune responses. Aluminum adjuvant, such as the aluminum hydroxide (Alum), remains to be the only adjuvant approved for human use in the U.S. It forms a precipitate when combined with soluble antigen, and the slow release of the antigen from the precipitate at the injection site causes prolonged, strong antibody responses [1,2]. However, Alum is a relatively weak adjuvant and has various limitations [2]. Therefore, there is a critical need to search for or to devise alternative vaccine adjuvants to improve the immune responses induced by recombinant protein antigens.

In recent years, particles of nanometer or micrometer scales are increasingly used as antigen carriers, and it is generally accepted that microparticles and nanoparticles have adjuvant activity [3–5], which is likely due to the easiness for antigen-presenting cells (APCs) to take up antigens associated with the particles, as compared to free antigens in solution. In theory, one expects that nanoparticles have a more potent adjuvant activity than microparticles. It was reported that particles with a diameter of 500 nm or less were optimal for uptake by APCs such as dendritic cells (DCs) and macrophages [6,7]. In addition, data from a recent study showed that small nanoparticles (20–200 nm) can freely drain to the lymph nodes (LNs) for antigen presentation, whereas DCs were required for the transport of large microparticles (0.5–2 μm) from the injection site to the LNs [8]. Therefore, it is likely that antigens associated with nanoparticles can reach the draining LNs for antigen presentation not only by the trafficking of APCs, but also by direct draining, which is expected to lead to a strong immune response. However, recent data from studies aimed at correlating the particle size of the antigen carriers and the resultant immune responses are rather controversial. For example, Wendorf et al. [9] reported that comparable immune responses were induced in mice by protein antigens (Env from HIV-1 and MenB from *Neisseria meningitides*) adsorbed onto anionic microparticles (~1 μm) and nanoparticles (110 nm) prepared with poly(lactic-co-glycolic acid) (PLGA) polymers. However, data from a study by Gutierro et al. [10] showed that when BSA was entrapped in PLGA particles of different sizes (200 nm, 500 nm, and 1 μm), the 1 μm microparticles generally induced a stronger serum anti-BSA IgG response than the 200 nm or 500 nm nanoparticles. Similarly, Kanchan and Panda [7] reported that the HBsAg entrapped in PLGA particles of 200–600 nm induced a weaker anti-HBsAg antibody response than HBsAg entrapped in PLGA particles of 2–8 μm, when injected intramuscularly into rats. On the contrary, data from many other studies showed that

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