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NEWS AND OPINIONS

Nanotoxoid vaccines

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Summary To improve innate defense against diseases, vaccine formulations are routinely administered to mount immune responses against disease-causing organisms or their associated toxins. These formulations are typically prepared with weakened forms of microbes, their surface proteins, or their virulence factors, which can train the immune system to recognize and neutralize similar infectious threats in later exposures. Owing to many unique properties of nanoparticles in enhancing vaccine potency, nanoscale carriers are drawing increasing interest as a platform for developing safer and more effective vaccine formulations. Notably, a nanoparticle-based strategy was recently demonstrated to safely deliver intact, non-denatured protein toxins to mount a potent anti-toxin immune response. A biomimetic nanoparticle cloaked in biological membranes was used to sequester membrane-active toxins. Upon interaction with the nanoparticles, the toxins become retrained and lose their toxicity as they are precluded from interacting with cellular targets. The resulting particle/toxin complex adopts a nanoparticulate morphology that facilitates the toxins' intracellular delivery. This sequestration approach has immense immunological implications owing to its ability in enabling structurally preserved toxins for immune processing. This technique offers opportunities in novel toxoid vaccine designs that promise more effective anti-toxin immune responses and contrasts the existing paradigm in toxoid preparation, in which toxins are antigenically altered to ensure virulence removal. The potent nanotoxoid formulations provide a viable anti-virulence measure in combating microbial infections that involve membrane-damaging toxins, including methicillin-resistant *Staphylococcus aureus* (MRSA) and Group A streptococcal infections.

Since the concept of immunization originated in 1796, numerous vaccines have been developed and proven successful in eradicating or reducing the occurrence of many life threatening diseases such as smallpox, measles, tetanus, and pertussis. The appeal of reinforcing our body's immune system to combat diseases has motivated ongoing vaccine research. Currently, many life-threatening public health threats, such as HIV, malaria, and MRSA infections, remain the focus of vaccine development, and emerging strategies are being explored for more effective immunization.

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Recent advancement in nanotechnology has drawn scientists and engineers to exploit nanoparticles to enhance vaccine technology. The synthetic flexibility of nanomaterials provides much versatility for vaccine designs, and many advantages of nanoparticle-based formulations have been demonstrated. For instance, numerous studies have shown that nanoparticles can carry multiple antigens to effectively stimulate the immune system via either sustained antigen release or multivalent antigen display to the immune system [1,2]. Particle stability and surface properties can also be modified to improve antigen transport to lymphoid organs and to antigen-presenting cells [3,4]. In addition, particulate delivery systems also allow antigens to be coupled with substances that stimulate immune responses to further improve their potency [5]. With the aid of advanced nanoparticle functionalization, we recently demonstrated that cytotoxic virulent antigens such as bacterial toxins can be sequestered by biomimetic nanoparticles cloaked in biological membranes. Upon nanoparticle interaction, intact, non-denatured toxins lose their motional freedom and are ''detained'' by the membrane-cloaked nanoparticles. These detained toxins are precluded from initiating their normal virulence mechanisms and can thus be safely delivered in vivo for effective immune processing [6]. The particular toxin-detainment strategy adds a new dimension to nanoparticulate vaccines, which had previously focused on applying nanoparticles as passive carriers for antigens with weakened immunogenicity. The toxin-nanoparticle complex (denoted nanotoxoid) has immense implications in the preparation of toxoid vaccines, which can be applied for the prevention and management of many bacterial infections.

Bacterial toxins alter the normal metabolism of host cells, and many protein toxins have been identified as the primary causative factors in infectious diseases. The role of toxins in infections has prompted the development of toxoid vaccines, which are inactivated forms of toxins that can be administered to mount an anti-toxin immune response. Conventional toxoid preparation methods involve protein denaturation through heat or chemical treatment for toxin neutralization, but these disruptive techniques unavoidably compromise the antigenic information in the toxin proteins, thereby necessitating a tradeoff between toxoid safety and efficacy. The shortfalls of denaturation-based toxoid preparation are evidenced in the decades-long effort in the development of α-hemolysin (Hlα) toxoid against *Staphylococcal aureus* infections, as early development of denaturation-based Hlα toxoid vaccines were marred by either residual toxicity or inadequate potency [7]. More recent efforts have focused on the development of non-toxic but structurally conserved toxin mutants using advanced biomolecular techniques. In particular, site-directed mutagenesis has been applied to produce toxin mutants with minimal antigenic alterations from the target toxins, thereby minimizing the tradeoff between safety and efficacy [8]. In our nanoparticle-detainment strategy, particle carriers are applied to intercept toxins' virulence mechanism, thereby enabling unaltered toxins to be administered for immune processing (Fig. 1).

By using Hlα as a model toxin, we have demonstrated successful toxin detainment with a red blood cell (RBC) membrane-cloaked nanoparticle platform, which consists of natural RBC membranes stabilized by 80 nm biodegradable poly(lactic-co-glycolic acid) (PLGA) polymeric cores. Unlike conventional nanoparticles that are passivated by hydrophilic polymers such as polyethylene glycol, the RBC membrane-cloaked nanoparticles are enclosed by a unilamellar biomembrane bilayer, which serves as a substrate for spontaneous toxin interactions. The membrane-targeting Hlα readily inserted into the stabilized RBC membranes and were sequestered by the stable particle structure. Each nanoparticle was found to adsorb dozens of toxin monomers, and toxin detoxification could be achieved in a facile and reliable manner by mixing the toxin with a sufficient number of nanoparticles [6]. The resulting nanotoxoid showed no observable toxicity. In contrast to the rapid detoxification via particle detainment, heat inactivation required at least 60 minutes of heating at 70 °C for toxin neutralization. As detained toxins retain their protein structure, mice vaccinated with particle-detained Hlα generated significantly higher anti-toxin immune responses as compared to those vaccinated with heat-denatured Hlα. Most impressively, mice receiving three weekly doses of particle-detained Hlα vaccine became completely immune to the toxin. High doses of Hlα that can cause serious tissue damages in non-vaccinated subjects did not inflict any observable effect in the vaccinated mice upon subcutaneous injections.

The biocompatible nature of RBC membranes and PLGA polymers allow the immune system to selectively process the toxin cargoes while ignoring the rest of the nanoparticle carrier. No anti-nanoparticle immune response was observed despite the high anti-toxin responses generated by the nanotoxoid. The biomembrane-coated nanoparticles also allow for the detainment of other membrane-active protein toxins. Successful neutralization of two other types of pore-forming toxins, an oligomerizing streptolysin-O from Streptococcus bacteria and a small peptide from bee venom, was demonstrated using the RBC membrane-coated nanoparticles in an earlier work [9]. Given the broad presence of membrane-damaging virulence factors in pathogenic microbes such as *Escherichia coli*, *Helicobacter pylori*, *Clostridium perfringens*, and *Bacillus anthracis* [10], our biomimetic nanoparticles offer a versatile platform for vaccine development against many infectious diseases. In addition to the membrane-coated exterior that serves to sequester pore-forming toxins, the nanoparticles possess other characteristic nanoparticulate properties that were conducive to the immune processing of the toxin antigens. For instance, owing to the nanoparticles' stability and small size, the particles were able to facilitate the antigen delivery to lymphatic organs such as the spleen and the lymph nodes [6]. The nanoparticle/toxin complexes also possess a particulate morphology that is more prone to cellular ingestion as compared to free proteins. This property allows toxin antigens to be efficiently taken up and metabolized by cells for immune processing. Along with the antigenically preserved toxin antigens, these other factors likely contributed to the enhanced antibody responses.

The ability to neutralize toxins via the detainment strategy also highlights the intricate biomolecular machineries behind the virulence mechanisms of protein toxins [11]. For instance, pore-forming toxins such as Hlα and streptolysin-O require membrane interactions and oligomerizing actions