The ferritin nanoparticle provides a flexible and immunogenic platform for advanced vaccine design and development.

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BACKGROUND: Endemic and emerging viruses continue to cause significant worldwide mortality and morbidity. Development of vaccines that elicit potent and protective immune responses against viral pathogens is complex, with multiple areas that require optimization. Eliciting protective immune responses is in part due to viral mechanisms that attenuate or distract human immune responses including viral sequence diversity, viral glycoprotein structural conformational changes, and viral glycan masking. Utilizing molecular information for the design of stable, homogenous vaccines, that have increased immunogenicity is a critical component in the design of rational next-generation vaccines. By using structural biology as a principal vaccine design tool, many viral evasion mechanisms can be targeted and eliminated. In addition, structural biology allows the precise design of viral nanoparticle vaccines such as ferritin nanoparticle vaccines. The ferritin molecule is a self-assembling protein, that forms a spherical nanoparticle. Viral antigens can be genetically linked to the ferritin monomers, followed by mammalian cell expression of a particle that is decorated with viral antigens on its surface. The viral antigens can be optimized through an iterative design process to elicit broad immune responses. The ferritin nanoparticle platform has been assessed in a set of phase I clinical studies for use against influenza, SARS-CoV-2, and Epstein Barr virus, by scientists at NIH and WRAIR. The WRAIR EIDB structural biology group uses X-ray crystallography and electron microscopy to structurally characterize viral proteins to provide high-resolution details that can be used for improved designs or to characterize the immune response following natural infection or vaccination in both animals and humans. Here we report the iterative structure-based design and pre-clinical assessment of ferritin nanoparticles against multiple pathogens including coronaviruses, influenza, flaviviruses and arenaviruses.

METHODS: Here we carried out structure-based design and characterization of a set of ferritin nanoparticle vaccine candidates. In this work, we carried out designs based on known viral proteins that contain antigenic sites of vulnerability. We targeted the (i) influenza receptor-binding domain (RBD) (ii) coronavirus spike glycoprotein, (iii) flavivirus domain III, and (iv) arenavirus GPC molecule, and using structure-based design, generated constructs that had the appropriate structure. Vaccine candidates were produced in mammalian 293F cells by transient transfection for 5 days, followed by affinity and size-exclusion chromatography. Vaccine candidates were then analyzed by negative-stain electron microscopy, dynamic light scattering, and antibody recognition

using biolayer interferometry. A subset of designs were assessed in mouse immunogenicity studies and asessed for immunogenicity against a broad panel of antigens and pseudoviruses to determine both the potency and breadth of the immune response.

RESULTS: The ferritin nanoparticle platform was used to generate a set of vaccine candidates designed around specific virus targets of interest. This included (i) mosaic influenza receptor-binding domain (RBD) ferritin nanoparticle (ii) coronavirus spike glycoprotein ferritin nanoparticle, (iii) mosaic flavivirus domain III nanoparticle, and (iv) Old-World and New-World arenavirus glycoprotein nanoparticles. In each of these four examples, ferritin nanoparticles were produced using antigens multiple strains within a family of viruses. Nanoparticle vaccines could be produced at reasonable quantities ranging from 1 - 20 mg of purified material from 1L of mammalian cell culture. In all cases, the purified protein was assessed for nanoparticle formation by negative-stain electron microscopy. In all cases, nanoparticles readily formed and were stable. In the case of the coronavirus spike nanoparticle immunogens, multiple stabilizing mutations were introduced to ensure the spike trimer formed and maintained a prefusion conformation. Vaccine candidates were also assessed for their ability to bind to a set of specific monoclonal antibodies to characterize their antigenic properties. In the four diverse virus groups including influenza, coronaviruses, flaviviruses, and arenaviruses, nanoparticles were produced that showed the appropriate antigenic profile. The mosaic Influenza ferritin nanoparticle, and the coronavirus ferritin nanoparticles were tested in multiple animal vaccination studies. In the influenza RBD-ferritin study, potent immune responses were elicited against a broad array of influenza viruses that span over 100 years of H1N1 human infecting virus diversity. In the case of the coronavirus Spike ferritin nanoparticles, these vaccines elicited potent immune responses against a broad array of SARS-like viruses.

CONCLUSION: Structure-based vaccine design coupled with the ferritin nanoparticle platform enabled the creation of multiple potent immunogens. Pre-clinical animal studies completed to date highlight the adaptability and consistency of the platform in eliciting robust and potent immune responses. The ferritin nanoparticle platform can be leveraged against diverse viral strains from multiple families. The design and strategies described here can be readily transferred to other endemic or emerging pathogen vaccine design.

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