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Self-Assembling Nanoparticles Usher in a New Era of Vaccine Design

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In this issue, Marcandalli et al. (2019) report a self-assembling nanoparticle bearing an antigen from respiratory syncytial virus. This is the first time the structure, stability, and adjuvanticity of an antigen have been rationally designed at the atomic level and incorporated in one vaccine.

When Edward Jenner in 1796 inoculated James Phipps with material derived from pustules of cows to make him immune to smallpox, he had little idea of what he was doing. He was just replicating the observation that a disease, transmitted from horses to the nipples of cows and then to humans, made people forever secure from infection with smallpox (Jenner, 1798). Since then, for more than 2 centuries vaccines have been developed empirically, by inoculating cultured microorganisms, or purified components of them, that had been inactivated, stabilized, and often combined with natural immunostimulants, acting as adjuvants. Thanks to these vaccines, our society has been able to conquer most of the infectious diseases that used to kill nearly half of children during the first 5 years of life, and life expectancy increased by more than 30 years during the last century. However, some infections have proven too difficult to address by the empirical approach. One of them is the disease caused by the respiratory syncytial virus (RSV), which affects most children worldwide and for which vaccine attempts consistently failed from 1967 to 2017. In this issue of *Cell*, the paper by Marcandalli et al. (2019) not only describes an effective vaccine against RSV but also marks the beginning of the end of the 2 centuries of empirical vaccinology by describing the first vaccine fully designed *in silico* to optimize immunogenicity, robustness, and adjuvanticity. The clear superiority of the resulting vaccine suggests that, in a broader context, empirical approaches should yield to structure-based rational design.

The long road from empiricism to full rational design of vaccine molecules started in late 1970s, when the recombinant DNA technology allowed the expression of hepatitis B particles in yeast and licensing of the first recombinant vaccine (Figure 1) (Valenzuela et al., 1982). This was followed by the engineering of the *Bordetella pertussis* chromosome by making two precise codon changes that enabled the bacterium to produce a fully immunogenic, but completely non-toxic, form of pertussis toxin that was the basis for the development of an acellular pertussis vaccine (Pizza et al., 1989). The third step toward rational vaccine design was the production of the fully folded human papilloma virus (HPV)

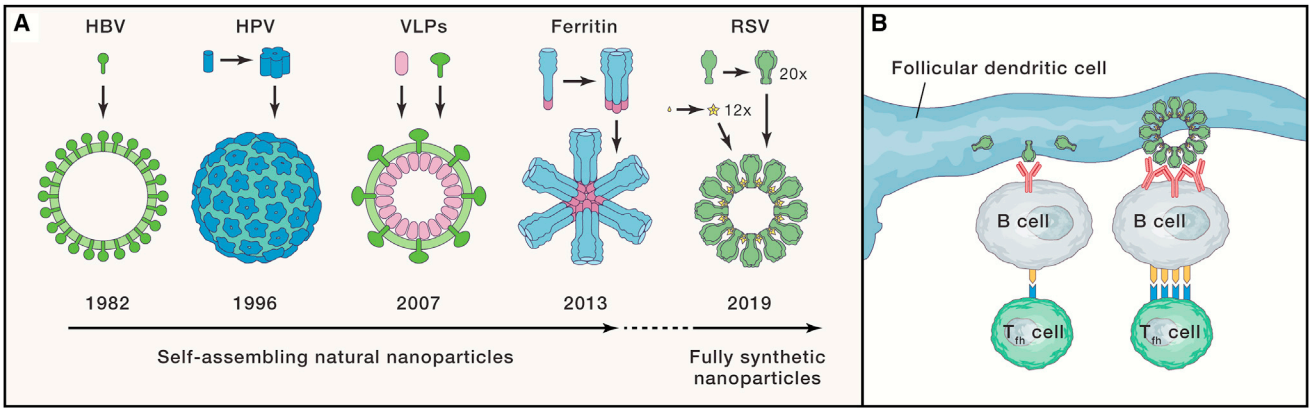

Figure 1. Nanoparticles in Vaccine Development

(A) A timeline depicts the evolution of nanoparticles in vaccine development. HBV, hepatitis B virus; HPV, human papillomavirus; VLP, generic virus-like particle; Ferritin, ferritin-based nanoparticle with influenza trimers; RSV: fully synthetic nanoparticle exposing 20 copies of the DS-Cav1 trimer of respiratory syncytial virus (Marcandalli et al., 2019).

(B) Antigen presentation by follicular dendritic cells of soluble (left) or nanoparticle (right)-based antigen, showing that the nanoparticle engages stronger interactions between antigen and B cell and between B cell and the follicular helper T cell (T_{fh}), resulting in stronger and longer-lasting immunity.

capsid in yeast or insect cells and the licensing of the vaccine against cancer of the cervix (Schiller and Lowy, 1996). Later, the advent of high-throughput sequencing and access to full pathogen genomes fostered a more rational selection of vaccine antigens and the licensure of the genome-derived meningococcus B vaccine (Pizza et al., 2000). A few years later, when the impact of high-throughput determination of the molecular structure of antigens became clear, it was predicted that a field of "structural vaccinology" would one day allow the complete rational design of vaccine molecules (Serruto and Rappuoli, 2006). The great progress in X-ray crystallography, cryoelectron microscopy, and computational protein design have made this dream a reality, exemplified by a new vaccine candidate against RSV described in this issue (Marcandalli et al., 2019).

Most candidate vaccines against RSV are based on the fusion protein (F), a trimer that in the prefusion conformation (PreF) is a good vaccine, while in the post-fusion (PostF) conformation, it is a poor vaccine. Unfortunately, the natural PreF is very unstable and adopts a PostF conformation very rapidly, thus making vaccine development very challenging. This problem was solved few years ago in the laboratory of Peter Kwong by determining the X-ray structure of the PreF conformation and using structure-based design to modify the sequence of the protein, introducing disulfide bridges, trimerization domains, and cavity-filling mutations in order to obtain DS-Cav1, a molecule that is locked in the PreF conformation. DS-Cav1 induces both in animals and humans ten times more neutralizing antibodies than the PostF (McLellan et al., 2013). Although the engineered DS-Cav1 antigen, which is presently in clinical trials, represents a great advance in the field, it may not be fully stable and also requires adjuvants to be an effective vaccine. Remarkably, Marcandalli et al. have been able to further engineer DS-Cav1 to make a computationally designed self-assembling PreF nanoparticle displaying 20 very stable trimeric PreF molecules. The self-assembling structure maximizes antigen density, representing an optimal strategy for antigen presentation. This results in an additional 10-fold increase in the neutralizing titer. In other words, the new vaccine has been rationally designed to incorporate into the same molecule all important properties of a vaccine, which are immunogenicity, stability, and adjuvanticity. Overall, in the new vaccine, a 10-fold gain in neutralizing antibodies has been achieved by structure-based design of the PreF trimer and an additional 10-fold increase added by the delivery of the antigen on a nanoparticle, for a total increase of 100-fold in neutralizing antibodies, compared to the native PostF soluble molecule.

It is not new that nanoparticles are much better than soluble antigens in inducing an immune response. The HBV and the HPV vaccines are excellent vaccines because they are composed of proteins that naturally assemble and form nanoparticles resembling the viral structures (Valenzuela et al., 1982; Schiller and Lowy, 1996). The superior immunogenicity of antigens exposed on nanoparticles compared to soluble single antigens can probably be explained by the fact that in the germinal centers they are able to engage multiple receptors of B cells, which can therefore retrieve and process larger amounts of antigen and have a stronger interaction with the follicular T helper cells (Figure 1B), leading to higher levels and longer duration of immunity. Recently, the increased ability to manipulate viral genomes has made it possible to take advantage of the natural property of viral proteins to assemble into nanoparticles, thereby incorporating many types of antigens into artificial viral-like particles (VLPs). In animal models, VLPs are capable of inducing excellent protection against several pathogens (Ludwig and Wagner, 2007). Monomers of self-assembling proteins such as ferritin, which form nanoparticles composed of 24 identical polypeptides, have also been fused to antigens such as influenza hemagglutinin (HA) to expose on the surface of the nanoparticles eight regularly spaced HA trimers. Such a

nanoparticle induces 10-fold-higher antibodies than licensed vaccines (Kanekiyo et al., 2013). Finally, the work by Marcandalli et al. represents a quantum leap for the nanoparticle concept, because instead of relying on molecules that naturally form nanoparticles, they fully designed from scratch the self-assembling proteins. The ability to fully design molecules without the need to use natural components frees vaccinology from mimicking nature. From here, the sky is the limit.

DECLARATION OF INTERESTS

The authors are full-time employees of the GSK group of companies.

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