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Mini Review

Self-assembling protein nanoparticles in the design of vaccines

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ABSTRACT

For over 100 years, vaccines have been one of the most effective medical interventions for reducing infectious disease, and are estimated to save millions of lives globally each year. Nevertheless, many diseases are not yet preventable by vaccination. This large unmet medical need demands further research and the development of novel vaccines with high efficacy and safety. Compared to the 19th and early 20th century vaccines that were made of killed, inactivated, or live-attenuated pathogens, modern vaccines containing isolated, highly purified antigenic protein subunits are safer but tend to induce lower levels of protective immunity. One strategy to overcome the latter is to design antigen nanoparticles: assemblies of polypeptides that present multiple copies of subunit antigens in well-ordered arrays with defined orientations that can potentially mimic the repetitiveness, geometry, size, and shape of the natural host-pathogen surface interactions. Such nanoparticles offer a collective strength of multiple binding sites (avidity) and can provide improved antigen stability and immunogenicity. Several exciting advances have emerged lately, including preclinical evidence that this strategy may be applicable for the development of innovative new vaccines, for example, protecting against influenza, human immunodeficiency virus, and respiratory syncytial virus. Here, we provide a concise review of a critical selection of data that demonstrate the potential of this field. In addition, we highlight how the use of self-assembling protein nanoparticles can be effectively combined with the emerging discipline of structural vaccinology for maximum impact in the rational design of vaccine antigens.

1. Introduction

Vaccines are among the most outstanding achievements in human medical history. Through their power to prevent or reduce the burden of infectious diseases they make an enormous global impact by improving the life quality of both humans and animals. Vaccines may save up to three million children's lives and up to six million total lives each year [1,2]. In addition to their contribution to an increased survival rate, vaccines are also an essential medical tool to protect against cancers and devastating sequelae derived from viral and bacterial infections, such as human papillomavirus (HPV) or meningitis, allergies, autoimmune diseases, or even drug dependencies.

However, there are many important pathogens against which vaccines do not yet exist, and some current vaccines could be improved. For example, some vaccines do not protect against all circulating strains of a pathogen because many microbes have developed sophisticated mechanisms to escape the host immune system. Mutations on the antigens of microbes such as influenza (flu), human immunodeficiency virus (HIV), or meningococcus constitute a rapidly changing 'disguise' to avoid recognition by trained immune cells that might otherwise prevent infection or disease. Further, some vaccine antigens do not elicit sufficiently durable or potent immunity. In addition to this, the rise of drug-resistant pathogenic entities such as those causing shigellosis demands our attention in the search for proficient vaccines [3]. Therefore, a major research focus is to seek ways to boost vaccine-induced host protection against pathogens, by developing novel antigens that evoke a more robust and protective immune response.

Many effective vaccines developed in the past used live-attenuated strains of a pathogen, or inactivated killed pathogens [4]. Live-attenuated vaccine strains are typically highly immunogenic, but carry inherent safety concerns, given the potential of these weakened viral particles to revert into disease-causing viruses. Additionally, mutagenic events within the host organism may generate more virulent strains. Conversely, while inactivated or killed vaccine pathogens cannot replicate nor revert into more virulent forms, they tend to stimulate a weaker immune reaction, and thus may require the administration of multiple dosages, an important practical limitation. An effective way to address these limitations has gradually emerged through studies of self-assembling proteins, which can be used as nanoparticles mediating multi-copy antigen display.

One of the earliest examples of a self-associating protein particle was reported in the 1950s: a protein extracted from the tobacco mosaic virus (TMV) was found to form rod-shaped particles, which morphologically

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resembled the original TMV but which did not contain genetic material [5]. Later, in the 1970s, the hepatitis B virus (HBV) surface antigen (HBsAg) was purified from infected human serum [6]. Electron microscopy (EM) and ultraviolet absorption studies revealed that HBsAg formed spherical particles with an average diameter of ~22 nm and which, importantly, like the TMV protein particle, lacked nucleic acid and hence were non-infectious. Preparations of such virus-derived 'nanoparticles' formed the first efficacious HBV vaccine, licensed in 1981 [7], and represented a milestone that created a new focus in the field of vaccinology. Indeed, antigen nanoparticles, first exemplified by HBsAg, have now emerged as a leading strategy in the development of safe and potent vaccines.

What are the advantages of nanoparticle antigens? Key parameters governing the elicitation of an efficient immune response to a microbe include both antigen density and distribution on the pathogen surface [8]. B- and T-cell stimulation and activation, and the subsequent secretion of antigen-specific antibodies by plasma cells, rely on effective cross-linking between B-cell surface immunoglobulins (the B-cell receptor, BCR) and the recognition pattern presented by the pathogen. The high density and structurally ordered antigenic array presented by a nanoparticle provides a molecular scenario where multiple binding events occur simultaneously between the nanoparticle and the host cell BCRs (Fig. 1). This multivalent molecular and cellular setting favors the fruitful network of stimulatory interactions, as opposed to the weaker effect of monovalent binding afforded by single soluble recombinant antigens. Indeed, the high avidity for the nanoparticle provided by the multivalent interaction constitutes a critical step in the induction of a potent immune reaction (Fig. 1). Because of these advantageous

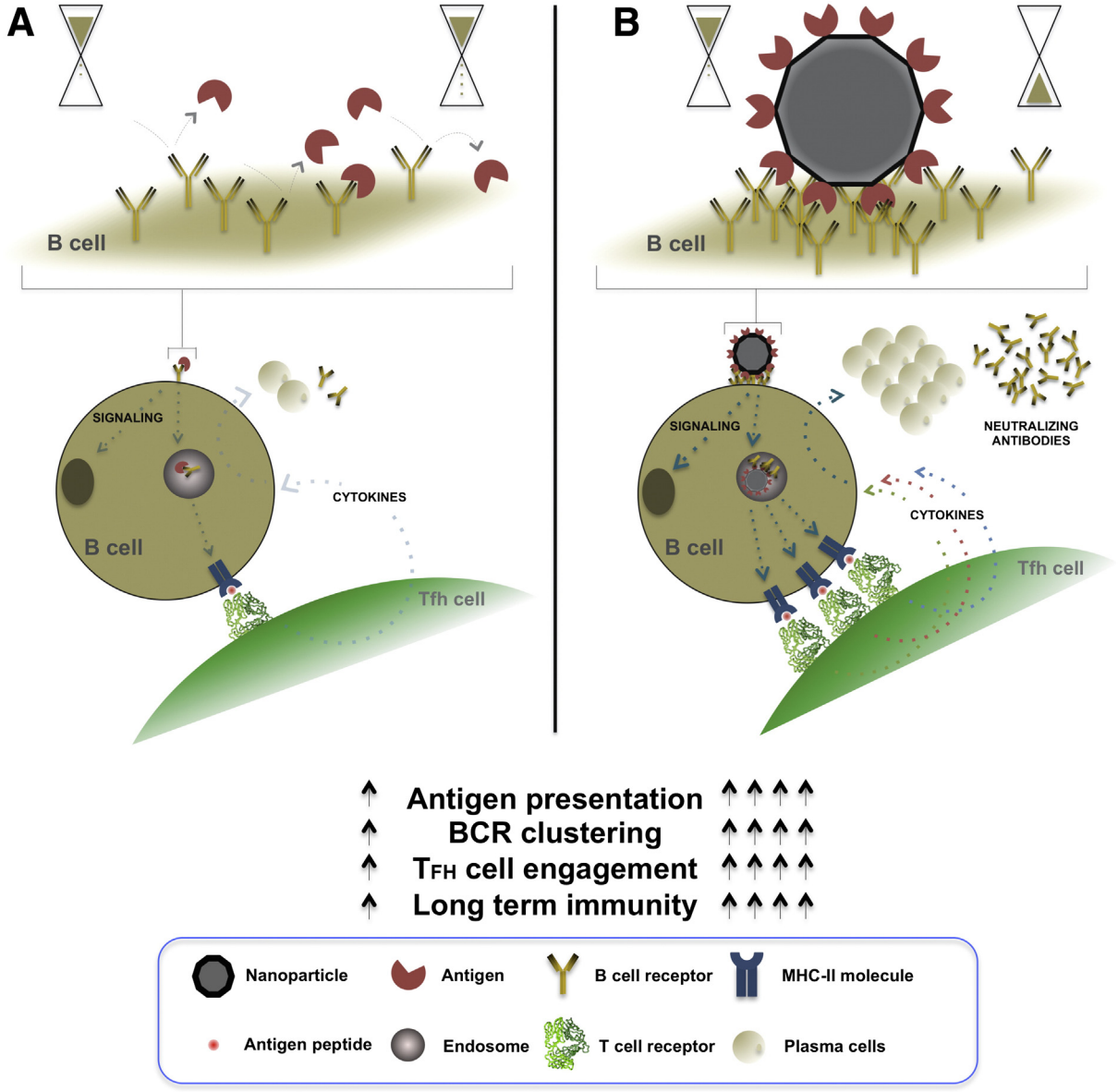

Fig. 1. Multivalent nanoparticles favor the generation of potent, long-lived immunoprotection in germinal centers. Recombinant nanoparticles loaded with the desired antigen are designed thoroughly to present multiple copies of a pathogen epitope in a highly ordered manner on the surface of a self-assembling nanoparticle. As opposed to single recombinant antigens that provide brief half-life 1:1 interactions with the BCRs (A), the polydentate nature, i.e. avidity, of the interaction with the nanoparticle enables tighter and prolonged bindings: the dissociation of one antigen molecule can be compensated by the binding of a new antigen molecule or re-association with a new BCR (B). This scenario enables the clustering of BCRs for multiple and simultaneous engagement with the antigen epitopes. Thus, the B-cell traps the antigen-loaded nanoparticle to establish a durable, localized and strong recognition that translates into B-cell intracellular signaling, internalization and processing of the antigen for presentation, via molecules of the MHC complex, to the T follicular helper cells (Tfh) within the germinal centers. This new recognition evokes the secretion of regulatory cytokines by the Tfh cell and ultimately the evolution of B cells into plasma cells that can secrete antigen-specific neutralizing Abs.