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Research update

Vaccine technologies: From whole organisms to rationally designed protein assemblies

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ABSTRACT

Vaccines have been the single most significant advancement in public health, preventing morbidity and mortality in millions of people annually. Vaccine development has traditionally focused on whole organism vaccines, either live attenuated or inactivated vaccines. While successful for many different infectious diseases whole organisms are expensive to produce, require culture of the infectious agent, and have the potential to cause vaccine associated disease in hosts. With advancing technology and a desire to develop safe, cost effective vaccine candidates, the field began to focus on the development of recombinantly expressed antigens known as subunit vaccines. While more tolerable, subunit vaccines tend to be less immunogenic. Attempts have been made to increase immunogenicity with the addition of adjuvants, either immunostimulatory molecules or an antigen delivery system that increases immune responses to vaccines. An area of extreme interest has been the application of nanotechnology to vaccine development, which allows for antigens to be expressed on a particulate delivery system. One of the most exciting examples of nanovaccines are rationally designed protein nanoparticles. These nanoparticles use some of the basic tenants of structural biology, biophysical chemistry, and vaccinology to develop protective, safe, and easily manufactured vaccines. Rationally developed nanoparticle vaccines are one of the most promising candidates for the future of vaccine development.

1. A basic overview of vaccine function

Vaccines are one of the greatest public health innovations in human history. Vaccination provides an extremely effective mechanism to deal with infectious diseases by preventing the development of morbidity and mortality. The World Health Organization estimates that vaccines prevent 2–3 million human deaths annually, and these numbers would rise by at least 6 million if all children received the recommended vaccination schedule [1]. Only two infectious diseases have been eliminated in human history, both the result of a successful vaccination campaign. The first, the human disease small-pox, was officially declared eliminated from the human population in 1979 [2]. The second, the livestock disease rinderpest was declared eliminated in 2011 [3]. While other diseases such as measles and polio are also close to elimination there is still much to be done [2].

Infectious disease vaccines work by serving as a prophylactic controlled exposure to an infectious agent. This initial exposure ideally induces a strong immune response in a vaccinated individual. A vertebrate's immune system is composed of two different branches, the innate and adaptive immune system. Following exposure to an infectious agent or administration of a vaccine, activation of the innate immune system precedes generation of adaptive immunity. The innate immune system is composed of a diverse array of cell types such as neutrophils, dendritic cells, monocytes, macrophage, and eosinophils all of which function to interact with foreign molecules in a nonspecific manner. Innate immune cells phagocytose infectious agents, secrete inflammatory cytokines, and/or attract and activate other immune cells through the secretion of chemical messengers such as chemokines. These processes lead to initiation of an effective immune response [4].

Vaccines are ultimately dependent on the development of an effective adaptive immune response. Broadly, adaptive immune responses are divided into two different categories, humoral and cellular. Cells of the adaptive immune system respond to specific regions of infectious agents known as epitopes. One or more epitopes are contained on a larger molecule known as an antigen. Humoral immune responses are dependent on the activity of antibodies, secreted glycoproteins from B cells that bind to specific

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epitopes. A naïve B cell contains B cell receptors on its surface, which vary in their specificities. Upon binding of the B cell receptor to a matching epitope, B cells can mature into plasma cells and begin to secrete epitope specific antibodies that will ideally lead to protection against infection [5].

Cellular immune responses are based on the action of T cells. All nucleated cells have on their surface Major Histocompatibility Complex Class I (MHC-I) molecules. When infected with an intracellular infectious agent, cells are able to present on their surface linear epitopes from those infectious agents complexed with MHC-I to alert the immune system of the infection. Cytotoxic T cells (T_C) that contain the matching T cell receptor are able to bind to the MHC-I presenting specific epitopes leading to the death of the infected cell [4].

One of the most important cell types in vaccine development are T helper cells (T_H). Antigen Presenting Cells (APCs) such as dendritic cells, macrophage, and B cells are able to phagocytize, process, and present $CD4^+$ epitopes in complex with Major Histocompatibility Complex Class II (MHC-II) on their surface. These epitopes stimulate $CD4^+$ T cells leading to their maturation into T_H cells. Active T_H cells are able to stimulate cells of both the innate and adaptive immune system through the secretion of cytokines. These cytokines are able to modulate the immune response leading to a stronger and more effective immune response. Based on the profile of the secreted cytokine responses they are either classed as T helper 1 response (T_H1) or T helper 2 response (T_H2). T_H1 responses favor the development of a cellular based immune response, while T_H2 responses favor the development of a humoral immune response. Traditionally, vaccine development has focused on the development of strong T_H2 responses, but currently a vaccine candidate that has a balanced T_H1/T_H2 response is considered optimal [5].

After activation, B cells, T_H, and T_C undergo proliferation to effectively deal with infection. In an ideal situation some of these cells persist after clearance resulting in the development of immunological memory. When a previously exposed host is exposed to an infectious agent again, antigen-specific immune memory cells are activated and proliferate faster and to a greater magnitude, leading to rapid clearance of the infectious agent and mitigation of disease. Strong and effective memory responses protect hosts against subsequent infections leading to lifelong immunity, the hallmark of an effective vaccine [5].

Vaccines not only work on the organismal level, but also on the population level. In the concept known as herd immunity if a certain fraction of the population is immune to an infectious agent the disease will have a very low likelihood of finding another naïve host and spreading. The number of people who need to be vaccinated for herd immunity varies from disease to disease, normally between 60 and 90%. It is extremely important because in any given population some vaccinated individuals will not develop protection based upon genetics, there will be individuals who cannot be vaccinated because of age or disease state, and there will be some unvaccinated individuals [6,7]. Herd immunity is the altruistic side of vaccination that will ultimately lead to the elimination of pathogens from either the human or animal population.

2. The origin of vaccines

By the 15th century there are documented attempts in Middle Eastern and Asian cultures to prevent small-pox infection by variolation. In these cultures, the pustules from patients with mild cases of small-pox were taken and dried, then used to scratch the surface of another patient's skin, or inhaled. It was a way to inoculate people against a more severe form of the disease. It was protective, with lower death rates than infection of a naïve person with the small-pox virus. The concept of variolation was brought back to Europe in 1718 by Lady Mary Wortley Montagu, the wife of the British ambassador to the Ottoman Empire. She saw the practice and had her children variolated to prevent them from becoming infected with small-pox [8].

Edward Jenner, a country doctor in late 18th century in England, made two key observations. The first was that milkmaids previously infected with cowpox, a zoonotic disease that is easily transmitted from cows to humans, did not develop smallpox. He also noted that when he variolated patients who recovered from cowpox they did not develop a response of a typical small-pox lesion. He reasoned that by inoculating people with the material contained in cow-pox pustules he would protect them against subsequent infection with small-pox. He performed the first known vaccine trial in 1796 by taking cow-pox pustules from a milkmaid, and inoculating an 8-year-old boy. He noted that boy felt general malaise for a day, but recovered quickly. He later variolated the child with small-pox, however, the child did not show signs of becoming infected with the disease [9].

While somewhat controversial in his time Jenner spent the rest of his life publicizing his technique. At this point the germ theory of disease had not been established, and people did not understand that both small-pox and cow-pox were caused by closely related viruses. Many people had concerns that vaccination with a different disease would not actually lead to protection. It was not until 1837 when England began keeping Cause of Death Records, that William Farr was able to determine that communities that have had high vaccination rates had low rates of death from small-pox. Ultimately, in 1840 variolation was banned in England and vaccination became the standard prophylactic treatment for small-pox [8,10]. Jenner had succeeded in the development and implementation of the world's first vaccine (Fig. 1, Table 1).

French Microbiologist and Chemist Louis Pasteur made the next major advance in the development of vaccines. In 1879 while studying chicken cholera, *Pasteurella multocida*, he had chickens inoculated with a month old culture after a vacation. The inoculated chickens developed minor symptoms of the disease, but recovered. He later inoculated the same chickens with a fresh culture of bacteria and saw that chickens previously inoculated with the old culture were protected from infection, while naïve birds still developed symptoms [10]. Pasteur had stumbled onto the concept of attenuation. If microorganisms are grown in suboptimal conditions, or are treated with certain chemicals they are not as virulent as microorganisms grown under ideal conditions. By exposing the chickens to the attenuated bacteria Pasteur was able to induce protection against subsequent lethal challenge with the virulent *P. multocida*. In 1881 Pasteur was able to repeat similar findings by attenuating *Bacillus anthracis* and vaccinating farm animals with the attenuated *B. anthracis* [11].

In 1879 Pierre Galtier had discovered that something in the saliva of rabid dogs caused rabies in other mammals. By 1884 Pasteur had developed a way to propagate the infectious agent, decreasing the incubation time to days instead of months. He demonstrated that by inoculating dogs and other mammals with his attenuated strain of the rabies virus the animals were protected when challenged with the normal rabies virus. When 9 year old Joseph Meister was bitten by a rabid dog in 1885 Pasteur was able to vaccinate him with the attenuated virus preventing the boy from developing rabies [11] (Table 1). Pasteur was able to demonstrate that an attenuated infectious agent could still result in protection of vaccinated humans against subsequent exposure to the virulent infectious agent. His work led to the development of attenuated vaccines for typhoid fever, cholera, and plague in the late 19th and the early 20th century [10] (Fig. 1).