Chapter 18
Dosing Strategies of Nanovaccines

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18.1 Introduction

Vaccines are a complex mixture of biological substances. Common antigenic components of vaccines include proteins derived from viruses, bacteria and others: live attenuated viruses; killed or inactivated viruses; bacterial toxins such as components of tetanus toxin (TT) and diphtheria toxoid (DT); toxins produced by bacteria such as pertussis component; genes encoding viruses known as deoxyribonucleic acid (DNA) (viruses); yeast cells; and other microorganisms that produce toxins that can cause diseases (Vaccine Types | NIH, 2022; Wilson-Welder et al., 2009). Antigenic components of vaccines can contain all or part of any pathogen, but most vaccines have common vaccination strategy components; for example, they are often made from a combination of multiple antigens into one vaccine. Vaccines can protect against diseases (Pollard & Bijker, 2020). They do this by exposing the body to a portion of a virus or bacterium to encourage the immune system to make antibodies and fight off illness. Vaccines may also contain other components such as inactive ingredients, preservatives and stabilizers (Types of vaccines, 2022) (Fig. 18.1).

Biological molecules for treating cancer, inflammatory and infectious diseases and autoimmune diseases are generally derived from living organisms or produced by recombinant DNA technologies. Several types of molecules can be classified as

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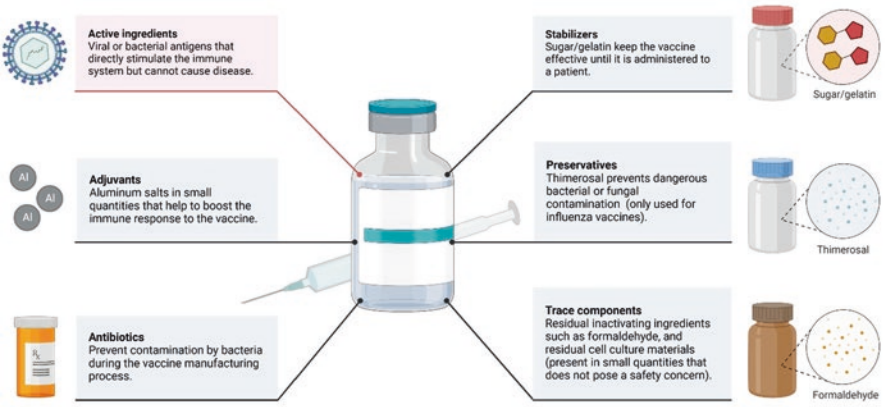

Fig. 18.1 Various components of the vaccine

biopharmaceuticals, along with growth hormones and purified or recombinant proteins (Dimitrov, 2012). In 2020–2022, these biomolecules gained much attention because of their attractive characteristics, specificity and potent therapeutic effects, resulting in high clinical success rates for approved products and increased biomolecule development (Gupta et al., 2017). In addition, the pharmaceutical industry generates billions of dollars from expanding marketed biomolecules. Many changes have been made to ensure compliance with regulatory requirements while optimizing and securing the high-scale production of biomolecules. Today, virtually all preparations in the biopharmaceuticals field are administered via parenteral routes, making formulation a major challenge for scientists (Taylor, 2015). Many studies focused their research on developing technologies and formulation strategies to deliver these molecules by alternative routes of administration with special attention to the oral route, always keeping the main formulation objective, that is, to ensure stability while formulating, during storage and till the administration of the patient.

In addition to the complicated structure and fragility of biomolecules, oral administration of these molecules also results in low bioavailability. Several literature reviews have already demonstrated the oral bioavailability of therapeutic proteins. While conventional vaccines cover the whole body, nanovaccines could target an area within the body where a disease or an infection originated. They stabilize various therapeutic agents, including peptides, proteins and nucleic acids, reducing vaccine doses and preserving antigen integrity. It is sometimes possible to correct hydrophobic compounds' solubility in a solution using nanoparticle systems so that they are suitable for parenteral administration through nanoparticle systems.

In addition, particle systems may have several advantages in mucosal immunity, like antigen protection against gastric and intestinal degradation (enzymes and acids) (Rodger & King, 2000; Homayun et al., 2019; Moeller & Jorgensen, 2008). It may also regulate the types of immunity induced by antigens and act as a reservoir for their controlled release. There is a possibility of a depot effect with these particles. They prevent the vaccine from spreading the antigen to the surface of the

injection site and release it gradually, allowing the vaccine to reach the immune cells for a longer period. Nanoparticles (NPs) can enter cells via endocytosis since their size is similar to cellular components.

Vaccines made with nanoparticles cross-present antigens through class I of the major histocompatibility complex and stimulate both humoral and cellular immune systems. In addition, antigen-specific antibodies can be used to cover nanovaccine particles to deliver targeted vaccines. Studies have shown that macrophages and dendritic cells can readily uptake cationic nanoparticles due to their positive charges, which are opposite to those on the membrane of dendritic cells to their high efficiency; they outperform conventional vaccines (Sarkar et al., 2019). There is no need for peripheral dendritic cells to move NPs towards lymph nodes. A nanovaccine also has the advantage of being sprayed in the nose, making it more convenient. With NPs, vaccines can reach cells more quickly and sometimes reach cells 30 times faster than with the vaccines alone. Studies have shown that nanoparticles are more effective in absorbing nutrients than microparticles (Turnis & Rooney, 2010).

18.2 Vaccine Adjuvants

The adjuvant concept is more than 80 years old, with the first adjuvant present in human vaccines, an aluminium salt (aluminium potassium sulphate, also known as alum). A new vaccine technology has spawned whether adjuvants need to be included in a new vaccine. Adjuvants are substances that can enhance and modulate the immunogenicity of the vaccine antigen, but they do not contain antigenic material. They may be coupled with the antigen (inactivated adjuvants) or antigenic components such as proteins and salts (Bonanni & Santos, 2011; Strugnell et al., 2011; di Pasquale et al., 2015; Zepp, 2010).

The recent shortage of novel influenza A (H1N1) pdm09 vaccines following the US Food and Drug Administration (FDA)'s decision to exclude subunit and inactivated poliovirus vaccines as candidates for pandemic response vaccines (PRVs) caused considerable concern among public health officials and stakeholders (Hawken & Troy, 2012). Adjuvants can be inactivated forms of older vaccines, such as alum, and newer substances, such as oils or squalene, called organic adjuvants. Different types of adjuvants and their uses in different vaccines are shown in Fig. 18.2**.**

18.2.1 Challenges of Oral Administration of Vaccines

Vaccines are one of the best ways to prevent disease, but they often fail. Nanotechnology is the most promising way to solve this problem because it allows us to create effective vaccines that can be given in a few doses and used for many years. Conventional vaccines are made by killing many cells and propagating them

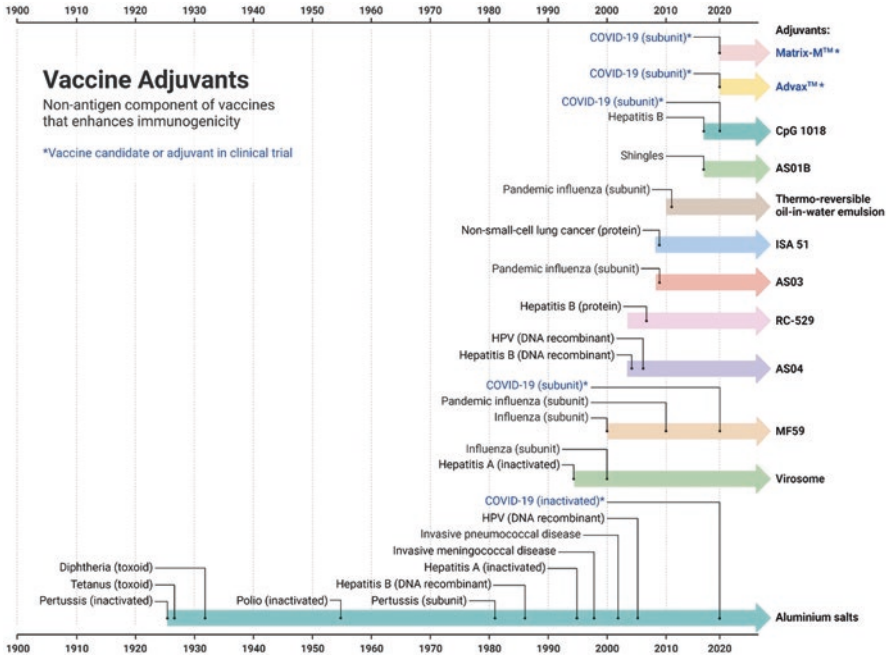

Fig. 18.2 Timeline of adjuvants used in human vaccines

in culture to generate antigens. Vaccine preparation depends on the host immune response to provoke an immune reaction, but not all strains induce antitumor and antibody responses equivalent or superior to the vaccines developed against wild-type viruses and bacteria. With the number of vaccines that need to be delivered at home, the need for setting up facilities with advanced technological infrastructure has been increasing (Lee, 2016).

Oral vaccines are the most convenient and available route of administration. The oral route is effective for delivering vaccines, which are normally biologically active substances produced locally in the mouth. Because of their convenience and practicality, they have become widely used in preventing infectious diseases. Oral vaccination strategies aim to prime the immune system by delivering subunit vaccines through many oral delivery systems. These vaccines can be divided into two broad categories: subunit vaccines and conjugate vaccines. Subunit oral vaccine formulations offer advantages over conventional parenteral routes in terms of safety and efficacy, ease of administration and convenience to patients. The most common products in this category are killed-virus-based vaccine formulations that include Ebola, Zika and MERS-CoV (Xie et al., 2020; Jhaveri & Torchilin, 2014; Hua, 2020; Alqahtani et al., 2021). Multiple routes can deliver oral vaccines against hepatitis C virus (HCV), but only a few of these are suitable for routine clinical use. The most popular route for delivering particles for proteinase K (PK)-resistant vaccine delivery is the oral route, although it has several drawbacks, including high pH, low

biological activity and dehydrating effects on both molecules and cells (Halliday et al., 2011). Among other strategies, it is necessary to consider the delivery system choice and its characteristics, including size, geometry, antigen loading and release kinetics capabilities and the ability to include functional molecules to improve their performance. However, there are several challenges to the oral administration of vaccines, such as transit time, safety and high cost. The primary challenge associated with administering vaccines is a lack of stability, making it difficult to administer to varying populations and high cost. Some controllable properties include size, geometry, antigen loading and release kinetic capabilities and finally the ability to include functional molecules to improve their performance (Vinarov et al., 2021). Tailoring these characteristics will prolong the residence time of immunogens, enable codelivery with antigens and adjuvants, boost their immunogenicity and target immune cells (specifically antigen-presenting cells [APCs]) for efficient transport, uptake and presentation. Passive vaccine delivery systems were developed to address these challenges. Microparticles and edible beads are used in passive vaccines (Coffman et al., 2010).

18.3 Nanotechnology and Nanovaccines

Nanotechnology has become a powerful tool in many fields of science since the discovery of electronics by Gabor in 1947. Nanoparticles carry many beneficial properties such as surface area, self-assembly and biointeractions. Nanotechnology compromises a material's size, shape and function using components with dimensions less than 100 nm in length, 50 nm in width and 1000 nm in thickness (Bayda et al., 2020). Nanoscale materials have many potential applications across industry and science, including within pharmaceuticals, cosmetics and fragrances. Nanotechnology allows scientists to create the world's smallest particles and make them last longer than normal. It has been used in vaccines and disease control, but some believe that this technology could produce drugs to fight cancer and other diseases (Boverhof et al., 2015; Jeevanandam et al., 2018).

Nanovaccines are being used in ways that could make an enormous difference to the health-care industry. Nanoscience and nanotechnology represent a revolutionary new field of medicine, and they have the potential to trigger a powerful immune response. Nanovaccines can combat diseases such as cancer and provide unique opportunities for treatment (Chauhan et al., 2020; Al-Halifa et al., 2019). For developing potential nanovaccines, researchers are looking at two different designs: the first technology is to use nanoparticles to carry an antigen (a particle attached to a protein), allowing it to be delivered through the bloodstream to body tissue at a higher rate than in conventional vaccines, and the second design is to use nanoparticles with attached antigens and deliver this directly through the bloodstream, bypassing any immune response. Nanoparticle-based vaccines have the potential to reduce lymphatic filtration, cause less tissue irritation and provide superior immunological memory than conventional vaccines (Reichmuth et al., 2016). Vaccine

delivery has historically been limited by a lack of a suitable vehicle for delivering nucleocapsid (NCt) nanoparticles.

Similarly, the natural lipids of the oleaginous mosquito egg yolk, lipid IVA and mixed esters of polyunsaturated fatty acids (MePS) are suitable vehicles for delivering NCT nanoparticles because they mimic the pH required for nanoparticle release and enhance corneal transfection due to their oil-like form and stability of pH. Delivering authentic NCT nanoparticles using an oleaginous vector demonstrated that nanoparticle-based vaccines are feasible and safe for humans. Future research will focus on identifying which nanoparticles are optimally suited for improving immune responses and generating antibodies (Mohan et al., 2013).

18.3.1 Nanovaccines and Their Applications

Tons of different formulations of nanovaccines have been developed, and synthetic nanoparticles stand out the most. The advantage of these nanoparticles is their ability to do more work than previous forms of vaccines (Pati et al., 2018), including (Fig. 18.3) the following:

1. They can deliver a significant number of molecules that can be targeted for recognition by the immune system.
2. They minimize undesired effects or side effects due to administration.

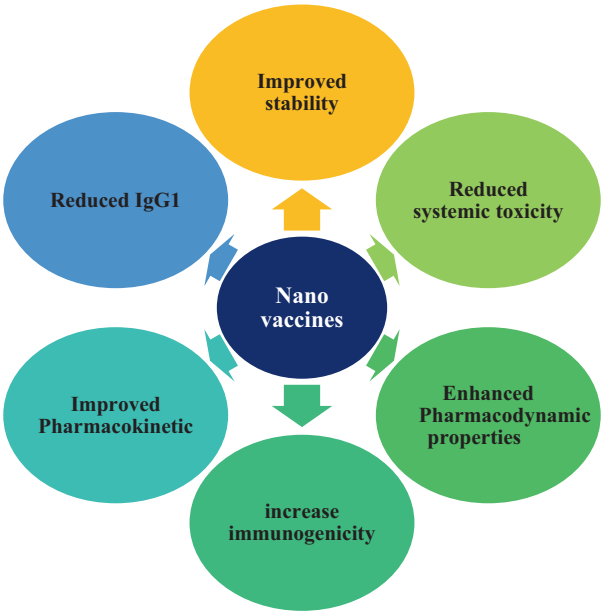

Fig. 18.3 Applications of nanovaccines

3. They have high selectivity and delivery efficiency as compared to traditional vaccines.
4. They deliver even greater numbers of molecules but are very small enough that they cannot elicit an immune response, and yet, they are still able to stimulate regulatory T cells (Tregs), which leads to better protection against infections.
5. Not only do these antibodies enhance and boost antibody production, but they also stop the overproduction of cytokines and cells in response to a viral infection, which results in faster recovery.

Nanoparticle vaccines are the latest evolution of vaccine delivery, opening up exciting new possibilities for the future of immunization. Nanoparticles are engineered to improve immunogenicity and decrease degradation by improving cross-linking, stabilizing antigen release, or adding an adjuvant effect.

The process of developing nanoparticle vaccines is similar to the method used for developing traditional vaccines, but the characteristics and design of these particles have significantly evolved. Nanoparticles deliver targeted genes to cancer patients through innovative and cheap drug delivery platforms (Diaz-Arévalo & Zeng, 2020). Nanoparticles are produced by chemically cross-linking protein antigens and carrier molecules to increase immunogenicity and decrease the degradation of the antigens. The properties of this vaccine include improved stability, reduced systemic toxicity, enhanced immune responses through IMMUNIN structural reversion (isolation of a single viral spike), reduced immunoglobulin G1 (IgG1) antibodies towards the carrier when exposed to an antigen and the ability to adsorb more antigens at the surface of nanoparticles than those in bulk (Kim et al., 2019; Yun & Cho, 2020).

18.3.2 Highlights of Polymeric/Particulate Vaccines

1. Polymeric/particulate vaccines are pharmaceutical products made from a non-protein subunit of the virus or bacterium and associated adjuvants. These can be either life, killed or inactive.
2. A polymeric/particulate vaccine contains both an antigen, a vehicle in which it is delivered to the immune system and a medium for delivering it. A polymer/particulate vaccine is made of a solid or liquid in the form of a micelle or hollow sphere, and then, the particles are released into the patient's body.
3. Particles can be any aspect of the formulation that makes up the vaccine, from encapsulated antigens to adjuvants like aluminium phosphate.
4. Polymeric vaccines contain antigenic proteins. The surface of the particulate carrier can be chemically modified to increase its immunogenicity.
5. Particulate vaccines are made from petroleum and particulate glass microfibres or synthetic polymer/tissue particles such as polylactide, poloxamer and povidone-K. Particulate vaccines have been developed to provide increased local immunity due to the long duration of immunity induced by the antibodies that bind to the antigen.

18.3.3 Single-Shot Vaccines

Single-shot vaccines result from engineering to catch diseases early and quickly. The vaccine takes on the illness and neutralizes it immediately without remembering to take another dose later. Single-shot vaccines have been live attenuated vaccines, killed vaccines and recombinant vaccines. The most common single-shot vaccine is H5N1 pandemic vaccine. The vaccine uses the knowledge that single-dose vaccines protect large mammals such as humans against infectious diseases caused by viruses. Single-shot vaccines, unlike multidose schedules, can be administered by a simple intramuscular injection. A novel Vernier pipette that allows accurate dispensing of precise volumes of liquids and lyophilization is a technology used to produce single-shot vaccines (Khademi et al., 2018). The production process of lyophilized vaccines, including developing a novel Vernier pipette, allows the accurate dispensing of the precise volume of liquids. The functional utility of lyophilized vaccines is often limited by low stability and the need to store them in a refrigerated environment. It utilizes the single-shot concept to produce the lyophilized vaccine product efficiently and quickly. Rather than relying on manual pouring techniques that are inefficient, time-consuming and prone to errors, this method relies on a novel Vernier pipette that allows precise dispensing (Bora et al., 2020).

18.4 Calculating Annual Vaccine Needs from the Size of the Target Population

The National Immunization Survey found that the population size of people who are 19 to 35 years old and have visited the doctor in the last year is 82 million. There are 86.6 million people aged 19–35 years who have seen a doctor in the past 12 months, and there are 92.5 million in this age group who have not been vaccinated against tetanus, diphtheria or pertussis (the three leading causes of a childhood illness) (Immunization Module, 2022) (Fig. 18.4).

The number of vaccines needed to achieve population coverage depends on the following two factors:

- The vaccine's age-specific target population.
- The total number of people required for a given population.

For example, if a vaccine is targeted for new-born immunization, all persons born in a given year must receive it during their first 18 months of life. Similarly, adults who have never received any vaccines will require a booster if they were born before 1980 and did not receive menses-based tetanus toxoid (Tdap) vaccination.

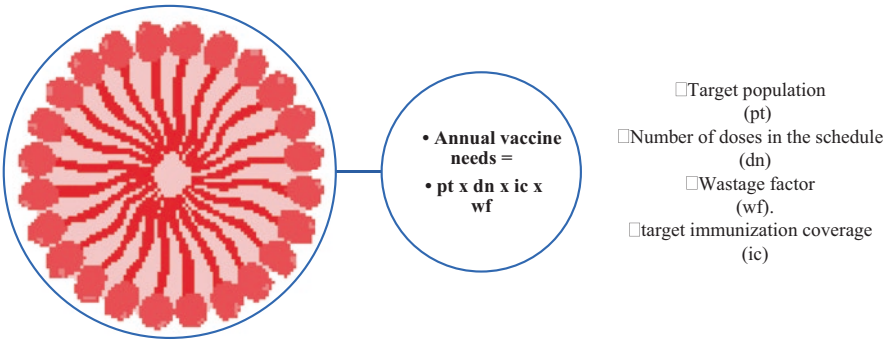

Fig. 18.4 Vaccine volume calculations

18.4.1 Determination of the Number of Doses for Nanovaccines

The number of doses included in a bank of vaccines can be determined by calculating the average number of doses contained in a set of medical supplies (Vu et al., 2021). The average number of doses supplied can be calculated using a simple procedure based on the following assumptions:

I. The ability of an individual to administer a certain dose is proportional to their weight or size.
II. No more than one dose may be available at any given time (either because there is only one unused dose or because it has been used).
III. It takes no more than 30 seconds per dose administered (this may be increased if the same needle is used repeatedly).
IV. Each person receives only one dose per month unless complications occur or there is some other reason for repetition.

Example To estimate the number of doses to include in a bank of vaccines, we need to know how many doses have been ordered and what their average price is. Consider a hypothetical vaccine with a list price of US$5 per dose. The manufacturer may estimate that 60% of children who receive the vaccine will be protected against disease, while 20% will not be protected. How much doses required will have to determine in a bank, multiply 60% by US$5/dose, plus 20% by US$5/dose, times 30%, which equals 13 times 20% equals 45 units × 3 units minus 18 units × 5 units equals 1.

18.5 Dosing Strategies and Their Importance

Nanoparticle-based vaccines represent one of the most important technologies in the emerging field of biodefense and personalized medicine. By using nanoparticles that are small enough to cross the blood–brain barrier, yet large enough to interact with the immune system, it may be possible to develop therapies against antigens that are able to elicit protective immunity without producing inflammation or neutralizing antibodies. The concept of dosing strategies for nanovaccines focuses on scientific and technological solutions for the preparation, size modification and future applications of nanovaccines. This is an important area as more focus is being directed towards nanoweapons by threat actors with increasing financial capabilities, speed and ease within which to synthesize compounds, manufacture them into weapons and dispense them. Nano criteria for dosing strategies of nanovaccines are more straightforward to enter into a computer than clinical doses. Safety concerns can be pursued with the traditional methodologies, but quantification of plasma levels comes at a high cost in terms of time and patient data collection (Zhou et al., 2020). The feasibility of delivering DNA-based vaccines within polymer nanoparticles has been demonstrated in rabbits, and information is available on the use of lipid nanoparticle–DNA conjugates as targeted delivery vehicles in mice. The ability to formulate a novel vaccine (containing an encapsulated antigen) that remains stable at high temperatures and pH has been developed (Semple et al., 2022). The nano criteria offer a powerful tool for improving vaccine delivery rapidly, specifically in situations where the scale, size or complexity of a trial is increased from clinical trials to large-scale trials on large populations. Nanocritic dose scaling reduces the costs associated with obtaining required information in the dosing regimen while improving safety standards and increasing efficiency. Smaller vials may allow for easier access to vaccine doses and more rapid delivery systems. Nanoparticle vaccines are also compatible with the current "cold chain" method of shipping vaccines, which involves refrigeration and holding at a temperature just above freezing (Semple et al., 2022; Liu et al., 2021).

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