Understanding modern-day vaccines: what you need to know

Volker Vetter\textsuperscript{a}, Gülhan Denizer\textsuperscript{b}, Leonard R. Friedland\textsuperscript{c}, Jyothsna Krishnan\textsuperscript{a} and Marla Shapiro\textsuperscript{d}

\textsuperscript{a}R&D Department, GSK, Wavre, Belgium; \textsuperscript{b}Regulatory Affairs Department, MSD, Brussels, Belgium; \textsuperscript{c}R&D Department, GSK, Navy Yard, Philadelphia, PA, USA; \textsuperscript{d}Department of Family and Community Medicine, University of Toronto, Toronto, Canada

\textbf{ABSTRACT}

Vaccines are considered to be one of the greatest public health achievements of the last century. Depending on the biology of the infection, the disease to be prevented, and the targeted population, a vaccine may require the induction of different adaptive immune mechanisms to be effective. Understanding the basic concepts of different vaccines is therefore crucial to understand their mode of action, benefits, risks, and their potential real-life impact on protection. This review aims to provide healthcare professionals with background information about the main vaccine designs and concepts of protection in a simplified way to improve their knowledge and understanding, and increase their confidence in the science of vaccination (Supplementary Material).

\textbf{KEY MESSAGE}

\begin{itemize}
  \item Different vaccine designs, each with different advantages and limitations, can be applied for protection against a particular disease.
  \item Vaccines may contain live-attenuated pathogens, inactivated pathogens, or only parts of pathogens and may also contain adjuvants to stimulate the immune responses.
  \item This review explains the mode of action, benefits, risks and real-life impact of vaccines by highlighting key vaccine concepts.
  \item An improved knowledge and understanding of the main vaccine designs and concepts of protection will help support the appropriate use and expectations of vaccines, increase confidence in the science of vaccination, and help reduce vaccine hesitancy.
\end{itemize}

\section{1. Introduction}

Vaccines are one of the greatest public health achievements of the last century and are estimated to save 2–3 million lives each year [1]. They have successfully eradicated smallpox and have greatly reduced the incidence of several major diseases such as polio and measles [1,2]. Licensed vaccines are now available to prevent over 30 different infectious diseases, several of which can be combined into single vaccines or administered at a single vaccination visit [1]. In this review, we highlight the different vaccine designs and illustrate them through various examples to give non-experts a basic understanding of vaccines and concepts of prevention.

\subsection{1.1. What is the aim of vaccination?}

The aim of vaccination is to induce a protective immune response to the targeted pathogen without the risk of acquiring the disease and its potential complications.

\subsection{1.2. How do vaccines work?}

Vaccines, like natural infections, act by initiating an innate immune response, which in turn activates an antigen-specific adaptive immune response [3]. Innate immunity is the first line of defence against pathogens that have entered the body. It is established within a few hours but is not specific for a particular pathogen and has no memory [4]. Adaptive immunity provides a second line of defence, generally at a later stage of infection, characterized by an extraordinarily diverse set of lymphocytes and antibodies able to recognize and eliminate virtually all known pathogens. Each pathogen (or vaccine) expresses (or contains) antigens that induce cell-mediated immunity by activating highly specific subsets of T lymphocytes and humoral immunity by
stimulating B lymphocytes to produce specific antibodies [3]. After elimination of the pathogen, the adaptive immune system generally establishes immunological memory. This immunological memory – the basis of long-term protection and the goal of vaccination – is characterized by the persistence of antibodies and the generation of memory cells that can rapidly reactivate upon subsequent exposure to the same pathogen [3].

1.3. What you need to know about vaccine design and concepts?

Vaccine design has made significant advances in the last century, evolving from serendipity to a more rational design due to advances in understanding immunological mechanisms and technology [1]. Depending on the biology of the infection and the disease to be prevented, a vaccine may require the induction of different humoral (i.e. antibodies) or cell-mediated (i.e. T cells) adaptive immune mechanisms to be effective. Understanding the mode of action of vaccines is therefore important to predict their efficacy, their safety profile, and their expected benefit for the vaccinated individuals and the general population.

Although vaccines are mostly seen as tools for individual protection, vaccines can also protect unvaccinated populations by reducing the rate of person-to-person transmission and limiting the risk for individuals to be exposed. This indirect protection, called herd or community protection, requires that a large portion of the population (75–95% depending on the disease), or a special group that plays a key role in transmission of the disease, is vaccinated [5,6]. Herd protection is often essential for the success of vaccination programs, such as for measles [7]. Similarly, vaccination of pregnant women can also indirectly protect infants in their first months of life through transfer of maternal antibodies from the mother to the foetus across the placenta [8]. This concept has been successfully established for tetanus, influenza and pertussis [8].

In contrast to the generally well-known pharmacological effects of different drugs, the differences between vaccine types are also important but less well understood by many healthcare providers. Different vaccines targeting the same pathogen can rely on very different concepts (Figure 1), each having advantages and limitations (Table 1). Choosing a particular vaccine therefore can depend on several factors such as the level of protection, the expected mode of action, the characteristics of the subject, or the disease elimination strategy. Many healthcare providers have not received sufficient education on vaccines, which could contribute to vaccine hesitancy among healthcare providers or patients [9].

2. Live attenuated vaccines

2.1. What are live attenuated vaccines?

Live attenuated vaccines contain pathogens that have been weakened, altered or selected to be less virulent than their wild-type counterparts. In their altered form, they cannot cause the actual disease or only mimic the disease in a very mild way.

Live attenuated vaccines are generally produced from viruses rather than bacteria because viruses contain fewer genes and attenuation can be obtained and controlled more reliably. The most common method to obtain live attenuated vaccines is to pass the virus through a series of *in vitro* cell cultures (e.g. in chick embryo cells) [10]. At each “passage”, the selected viruses become better at infecting and replicating in cell cultures but progressively lose their ability to infect and replicate in their original human host. Attenuation can also be achieved by low-temperature passages (e.g. 25 °C) [10]. This approach selects viruses that replicate well in a cold environment but less well at body temperature, therefore decreasing their pathogenicity in the human host.

Live attenuated vaccines act by causing a very limited type of infection. As the attenuated pathogen presents the same antigens as the original pathogen, healthy individuals develop immune responses comparable to those induced by the natural infection. Consequently, these vaccines induce robust cell-mediated and antibody responses and often confer long-term immunity after only one or two doses [11]. Another advantage of live viral vaccines, and a risk also, is that they can induce herd immunity through excretion of viral particles (i.e. viral shedding), which may indirectly “vaccinate” individuals living in the environment of the vaccine. This phenomenon differs from the herd protection induced by vaccinating enough individuals to stop transmission of the pathogen but may be also important to achieve a high protection level.

Live attenuated vaccines have some limitations. Although rare, clinical disease can occur after vaccination, but vaccine-induced symptoms are typically much milder than after natural infection. However, immunocompromised individuals are at risk of unregulated pathogen replication that may lead to severe infection or death. Therefore, live attenuated vaccines are often contraindicated in immunocompromised individuals, and these individuals should only receive
live attenuated vaccines after a critical benefit–risk assessment considering the type and severity of immunodeficiency. Similarly, live attenuated vaccines are also contraindicated during pregnancy due to the theoretical risk of foetal infection that may result in congenital disease (e.g. rubella or varicella) [12]. Finally, another consideration is that live attenuated vaccines have the potential to revert back to a form able to cause the disease, though this is very rare (e.g. oral polio vaccine [OPV]).

### 2.2. Which vaccines are live attenuated?

Many of the first vaccines that were produced consisted of live attenuated vaccines, such as rabies, smallpox, tuberculosis, yellow fever and OPV, some of which are still in use. A successful example is the yellow fever vaccine YF-17D. It was developed in the 1930s by attenuating a yellow fever virus strain by more than 200 serial passages through monkeys and cultures of mouse and chicken embryonic tissues [13].

All currently-used yellow fever vaccines derive from this attenuated strain [13]. In addition, YF-17D has been used to produce vaccines against two closely related viruses, Japanese encephalitis (IMOJEV, Sanofi Pasteur) and dengue (Dengvaxia, Sanofi Pasteur), by replacing the genes encoding the antigenic proteins by their equivalents [14,15].

Other classical examples of live attenuated vaccines produced by serial passage are those against measles, mumps, rubella and varicella, which are usually combined into trivalent (Priorix, GSK; M-M-RVAXPRO, MSD) or tetravalent (Priorix-Tetra, GSK; ProQuad, MSD) vaccines [16,17]. The live attenuated varicella vaccine strain is also used in the herpes zoster (shingles) vaccine (Zostavax, MSD) but this vaccine relies on a different rationale. It contains a high dose of the live attenuated varicella-zoster virus (>14 times more than in the varicella vaccine) to boost immune responses in older adults who are already immune to varicella to prevent viral reactivation and the development of shingles [18].
OPV is a live attenuated vaccine that was obtained through serial passages in non-human cells [19]. The attenuation of the three polio vaccine strains (Sabin 1, 2 and 3) greatly reduces their neurovirulence and transmissibility. OPV is easily administered through oral drops, inexpensive, and effective at inducing intestinal mucosal immunity [20]. Furthermore, OPV provides a high level of herd immunity through viral shedding and can be quickly and easily implemented in outbreak settings. However, in very rare cases (one case per million doses), OPV can mutate into a virulent form and induce very rare cases of vaccine-associated paralytic poliomyelitis [21].

### Table 1. Benefits and limitations of the different types of vaccines.

| Vaccine type                        | Benefits [27,68]                                                                 | Limitations [27,68]                                                                 | Examples                                                                 |
|-------------------------------------|---------------------------------------------------------------------------------|----------------------------------------------------------------------------------|------------------------------------------------------------------------|
| **Live attenuated vaccines**        | Mimic natural infection and immune response                                     | Contraindicated for immunocompromised individuals and pregnant women             | Measles, mumps, rubella, varicella (Priorix Tetra, ProQuad), rotavirus (Rotarix, Rotavac), herpes zoster (Zostavax), influenza (Flumist), oral poliovirus (OPV), yellow fever (Stamaril) |
|                                     | Elicit both antibodies and cell-mediated immunity                               | Less stable over time, heat labile                                              |                                                                       |
|                                     | Life-long immunity possible after 1 or 2 doses                                  | Possibility to reverse to natural form (e.g. poliovirus)                        |                                                                       |
| **Inactivated vaccines**            | Robust technique for production                                                | Limited immunogenicity, adjuvants may be required                               | Whole-cell pertussis (Tritanrix), hepatitis A (Havrix, Vaqta), rabies (Rabipur), tickborne encephalitis (Enceph), Japanese encephalitis (Ixiaro), cholera (Dukoral) |
|                                     | High stability                                                                  | Multiple primary and booster doses required to obtain long-term protection      |                                                                       |
|                                     | Impact on carriage                                                             | No replication of the inactivated pathogen                                       |                                                                       |
|                                     | Not contraindicated in immunocompromised individuals and pregnant women        |                                                                                  |                                                                       |
| **Split and subunit protein vaccines (natural or recombinant)** | Non-infectious                                                                  | No or limited innate defence triggers                                          |                                                                       |
|                                     | Low reactogenicity                                                             | Often have reduced immunogenicity compared to whole pathogen vaccines          |                                                                       |
|                                     |                                                                                  | For those vaccines with lower immunogenicity, adjuvants are often needed       |                                                                       |
| **Toxoid vaccines**                 | Vast experience as mature technology                                            | Vaccines only target the toxin and do not prevent infection by the pathogen   | Influenza (Fluarix, Fluarix Tetra, Fluvalav, Intanza, Vaxigrip, etc.), acellular pertussis, hepatitis B (Engerix B, Recombivax HB), human papillomavirus (Cervarix, Gardasil, Gardasil 9), meningococcal B (Bexsero, Trumenba), malaria (Mosquirix), herpes zoster (Shingrix) |
|                                     | Non-infectious                                                                  | No herd protection                                                              | Tetanus, diphtheria, acellular pertussis (as part of DTaP combination vaccines: Boostrix, Infanrix, Adacel, etc.) |
|                                     | Used as carrier proteins due to good immunogenicity                             | Priming and boosting necessary                                                  |                                                                       |
| **Polysaccharide vaccines**         | Easily identifiable target                                                     | Weakly immunogenic, elicit only a transient antibody response giving a limited duration of protection | Pneumococcal polysaccharide vaccine (Pneumovax 23), meningococcal polysaccharide vaccine (Mencevax) |
|                                     |                                                                                  | Limited immunogenicity in infants                                               |                                                                       |
|                                     |                                                                                  | Hyporesponsiveness after repeated doses                                          |                                                                       |
|                                     |                                                                                  | Limited or no impact on carriage                                                |                                                                       |
| **Polysaccharide conjugate vaccines** | Improved memory responses leading to increased protection in infants           | Booster doses may be required to attain long-term protection                    | Meningococcal C (Neisvac), A (MenAfriVac), ACWY (Nimenrix, Menveo, Menactra), pneumococcal conjugate vaccine (Prevnar, Prevnar 13, Synflorix), Haemophilus influenzae type b (ACT-HIB) |
|                                     | Provide a longer duration of protection than polysaccharide vaccines due to B- and T-cell responses |                                                                                  |                                                                       |
|                                     | Impact on carriage and transmission                                            |                                                                                  |                                                                       |
| **Reassortant live attenuated**     | Benefit from a live infection with a non-pathogen strain                       | Contraindicated for immunocompromised individuals                               | Rotavirus (RotaTeq)                                                   |
|                                     | Cannot cause the original disease, good safety profile                         | Risk of re-reassortants, especially when combination of several reassortants [69] |                                                                       |
|                                     | Improved tolerability due to combination of low pathogenic virus with antigen from high pathogenic virus | Immunogenicity limited to selected antigens                                      |                                                                       |
its use is incompatible with the final stages of polio eradication (i.e. eradication of both wild and vaccine-derived polio) and should be stopped after wild poliovirus transmission has been controlled. For this reason and to maintain population immunity, OPV has been replaced by an inactivated polio vaccine (IPV) in the United States, Europe, and an increasing number of countries worldwide [22]. However, IPV is less effective than OPV at inducing intestinal mucosal immunity among previously unvaccinated individuals and is less effective at inducing herd immunity via viral shedding [20]. Thus, as recently highlighted by the World Health Organization, both vaccines (OPV first and then IPV) are crucial for the eradication of polio [20].

Live attenuated vaccines targeting the same pathogen can also rely on different designs. This is the case for vaccines against rotaviruses, a major cause of acute gastroenteritis in children. Rotarix (GSK) is a single-strain human rotavirus live attenuated vaccine mimicking natural infection, whereas RotaTeq (MSD) is a chimeric pentavalent human-bovine reassortant vaccine, in which a nonpathogenic bovine rotavirus strain provides the backbone to express the antigenic proteins of the five most frequent human rotavirus serotypes [23].

Attenuation by cold-adaptation of the original pathogen is used for live attenuated intranasal influenza vaccine (FluMist, Fluenz, MedImmune). It is produced by inserting the genes of the antigenic proteins from circulating influenza viruses in the backbone of an attenuated cold-adapted influenza virus. Intranasal administration of this vaccine mimics the natural route of influenza infection and induces an immune response directly in the respiratory tract [24].

The only live attenuated bacterial vaccine currently in use is the bacillus Calmette-Guérin (BCG) vaccine, which was developed almost a century ago and is still the only licensed vaccine against tuberculosis [25]. This vaccine contains a bovine Mycobacterium bovis bacillus strain that had been attenuated by cell culture passage over several years [26].

3. Non-live vaccines

Non-live vaccines do not contain any living or infectious particles, so they cannot cause disease and cannot reactivate. Therefore, they generally have a good safety profile, even in immunocompromised individuals. However, a drawback of these vaccines is that immunogenicity and duration of protection tend to be less than for live vaccines, and they may require several doses or adjuvants to improve immunogenicity [27]. Therefore, these vaccines are usually given repeatedly based on the prime-boost principle to induce long-term immunity. This strategy, used since the advent of vaccines, aims at boosting antibody and cell-mediated immune responses through multiple primary doses and regular boosters. Impact on asymptomatic nasopharyngeal carriage of the pathogen and, consequently, herd protection by interrupting transmission can be achieved with some non-live vaccines. Non-live vaccines can contain inactivated whole pathogens or only parts of them such as proteins or polysaccharides (subunit vaccines).

3.1. What are inactivated vaccines?

Vaccines based on inactivated pathogens are produced by inactivating preparations of whole pathogens by heat, radiation, or chemicals such as formalin or formaldehyde. Inactivation destroys the pathogen’s ability to replicate and cause the disease but maintains its immunogenicity, so that the immune system can still recognize the targeted pathogen.

Inactivation approaches were first used to create vaccines against pathogens such as typhoid fever, plague and cholera [1]. Current examples of inactivated vaccines include the previously mentioned IPV, whole-cell pertussis, rabies and hepatitis A vaccines [1,28]. Whole-cell pertussis vaccines are produced locally in many countries using different methods; thus, they are heterogeneous and may elicit different immune responses [29]. In industrialized countries, they have been frequently replaced by the less reactive acellular vaccines based on a small number of selected antigens of the pathogen.

3.2. What are subunit vaccines?

Subunit vaccines contain selected fragments of the pathogen as antigens instead of the whole pathogen. These fragments can be proteins, polysaccharides, or parts of a virus that may form virus-like particles (VLPs). Subunit vaccines generally cause less adverse reactions than live or inactivated whole-organism vaccines, but they may be less immunogenic because they contain fewer antigens and the purification process often eliminates components that trigger innate immunity [30]. Examples of subunit vaccines include tetanus toxoid, inactivated split and subunit seasonal influenza, acellular pertussis and pneumococcal polysaccharide vaccines.
3.3. What are the different types of subunit vaccines?

3.3.1. Protein vaccines

Antigenic proteins can be purified from preparations of the whole pathogen, as for the acellular pertussis vaccines [31], or can be produced by recombinant genetic engineering [32]. In the latter case, a gene encoding the antigenic protein is inserted into an expression system able to produce large quantities of the antigen in cell cultures.

Since the 1970s, most influenza vaccines have consisted of split viruses deriving from preparation of inactivated influenza vaccines. Inactivated influenza vaccine production is a long process relying on embryonated chicken eggs, except for Flucelvax (Seqirus), which is grown in mammalian cell culture [33]. These split vaccines are then prepared by chemical disruption of the viral lipid envelope and, therefore, the viral particle organization. These vaccines retain immunogenicity but induce fewer adverse reactions than whole-virus inactivated influenza vaccines. Seasonal influenza vaccines contain in general three or four viral strains to cover the influenza A and B strains predicted to circulate during the forthcoming influenza season. A good match between the vaccine strains and the circulating strains is crucial to achieve high protection rates [34]. Influenza vaccination is therefore recommended annually because the viruses in circulation frequently change and because protective immunity is of short duration.

Acellular pertussis vaccines are other examples of purified antigenic proteins. These vaccines contain between one and five highly purified pertussis antigens, compared to more than 3000 antigens for whole-cell inactivated pertussis vaccines [31,35]. They have been introduced in many industrialized countries after concerns about whole-cell pertussis tolerance profile arose in the population, leading to lower vaccination coverage. Recently, a resurgence of pertussis has been observed in many regions of the world despite high acellular pertussis vaccine coverage. Although the reasons for this resurgence are complex, the shorter duration of protection and the probable lower impact of these vaccines on infection and transmission might play a role [31].

An example of recombinant protein vaccine is provided by the widely used hepatitis B vaccine in which the gene of the hepatitis B surface antigen (HBsAg) has been inserted into appropriate vectors for production in yeast (Engerix-B, GSK; Recombivax-HB, MSD) or mammalian cells (GenHevac-B, Sanofi Pasteur) [36]. The resulting recombinant protein is then purified.

The concept of combining recombinant proteins helped to develop the first malaria vaccine (Mosquirix, GSK). In this vaccine, the gene of a surface protein of the infectious form of *Plasmodium falciparum* is fused to the HBsAg gene, and the resulting recombinant fusion protein is expressed in yeast with free recombinant HBsAg [37]. These proteins spontaneously assemble into non-infectious VLPs (see Section 3.3.3) and are combined with an Adjuvant System (AS) to allow protection. The vaccine induces antibodies against parasites that have reached or are in transit to the liver (where they mature and multiply), thereby limiting the ability of the parasites to cause clinical disease. Due to the high frequency of malaria in children in some parts of the world, this vaccine has the potential to prevent a large number of malaria cases [37,38].

The advantages of protein vaccines have been used to develop a new subunit vaccine against herpes zoster (Shingrix, GSK). This vaccine, containing a recombinant varicella-zoster virus protein and an AS, has the objective of maintaining high efficacy also in older adults who are at higher risk of herpes zoster [39,40]. It is also being studied for use in immunocompromised individuals for whom live attenuated vaccines are often not recommended.

Finally, reverse vaccinology is a new technology in which genes encoding potential antigenic proteins are identified from the entire genome of a given pathogen [41]. The identified proteins are then tested *in vitro* and *in vivo* to determine whether they are immunogenic and induce protective antibodies. Reverse vaccinology has been used to develop a vaccine against the challenging *Neisseria meningitidis* serogroup B [42]. Unlike other *N. meningitidis* serogroups, serogroup B is covered by capsular polysaccharides that have similarities to human polysaccharides. This property substantially reduces the immunogenicity of these polysaccharides and could, at least theoretically, trigger antibodies against the human host and cause auto-immune diseases [42]. In addition, a vaccine relying on recombinant proteins has been unsuccessful because of the high antigenic variation in circulating strains [42]. Reverse vaccinology helped identify four novel antigenic proteins, which have been combined in a tetravalent meningococcal B vaccine (Bexsero, GSK) [42]. By contrast, a more traditional approach of protein screening was used to develop a bivalent meningococcal B vaccine (Trumenba, Pfizer) [43].

3.3.2. Toxoid vaccines

Some bacteria such as *Clostridium tetani, Clostridium difficile* or *Corynebacterium diphtheriae* cause disease...
by releasing pathogenic toxins. Vaccines against these diseases are produced by detoxifying the toxin using heat, chemicals (e.g., formaldehyde) or both. The inactivated toxins, called toxoids, are no longer pathogenic but retain their ability to induce toxin-neutralizing antibodies. Classical examples of toxoid vaccines are those against diphtheria and tetanus, which have been used since their discovery in the 1920s [44–46]. Pertussis toxoid is also included in all acellular pertussis vaccines [31]. Due to their good immunogenicity, toxoids are also used as carrier proteins for conjugate vaccines (see section 3.3.5) [47,48].

Toxoids provide protection through the induction of antibodies that must be present at disease onset to be effective. For this reason, toxoid vaccines require multiple doses to maintain adequate life-long protection. However, toxoids protect only against disease pathogenesis in vaccinated individuals but do not prevent infection or transmission [49]. Consequently, all individuals need to be vaccinated regularly, and indirect protection of unvaccinated people is generally not possible. This is notably illustrated by the tetanus vaccine. Tetanus is not transmitted from person-to-person but occurs through contamination of wounds with C. tetani spores that are widespread in the environment such as in the soil [45]. Therefore, high vaccination coverage does not provide herd protection and unvaccinated or individuals not receiving regular booster doses are potentially at risk.

3.3.3. VLPs

VLP vaccines are based on the observation that expression of certain viral proteins leads to the spontaneous assembly of particles structurally similar to the original viruses [50]. VLPs are not infectious because they lack the viral genome. However, the native conformation of the antigenic proteins is well preserved, which improves their immunogenicity compared to free proteins.

The best-known examples of VLPs are the human papillomavirus (HPV) vaccines that protect against cervical cancer caused by these oncogenic viruses [50]. Currently, licensed HPV vaccines contain antigenic proteins from HPV types responsible for most cervical cancers (e.g., Cervarix, GSK; Gardasil 9, MSD).

3.3.4. Polysaccharide vaccines

Streptococcus pneumoniae, Haemophilus influenzae type b and N. meningitidis are three encapsulated bacteria that cause severe invasive disease. They possess polysaccharide capsules that facilitate bacteria’s survival when carried in the nasopharynx and in the blood during disease pathogenesis [47]. First-generation vaccines against these pathogens were based on capsular polysaccharides purified from whole pathogens, such as the 23-valent pneumococcal polysaccharide vaccine that was licensed in 1983 (Pneumovax 23, MSD; Pneumo 23, Sanofi Pasteur).

However, polysaccharide vaccines are poorly immunogenic, provide only short term protection, and can lead to a reduced immune response after repeated vaccinations (i.e., hyporesponsiveness) [51,52]. Indeed, capsular polysaccharides are large molecules with repeated antigens that can be recognized by B cells leading to their direct activation without the need of T-cell help. Furthermore, polysaccharide vaccines are largely ineffective in children <2 years of age who are at high risk for invasive disease by these pathogens due to the immaturity of their immune system [51].

3.3.5. Polysaccharide conjugate vaccines

Studies performed in the 1920s–1930s showed that the immunogenicity of purified polysaccharides could be enhanced by coupling (i.e., conjugating) them to a protein [47]. This breakthrough allowed the development of second-generation vaccines against H. influenzae type b, S. pneumoniae and N. meningitidis serogroups A, C, W and Y that are effective in infants [47]. Conjugation transforms the T-cell-independent response induced by polysaccharides into a T-cell-dependent response that induces high-affinity antibodies and immune memory [47]. In addition, in contrast to polysaccharide vaccines, conjugate vaccines can induce herd protection by reducing asymptomatic nasopharyngeal carriage and, therefore, transmission of these pathogens [47].

Conjugate vaccines are produced by chemically linking polysaccharides to a carrier protein, which makes the production of these vaccines more complex. Different carrier proteins have been used, such as tetanus and diphtheria toxoids, the nontoxic variant of diphtheria toxin CRM197 (isolated from C. diphtheriae C7 [B197] cultures), the outer membrane protein complex from N. meningitidis, or non-typeable H. influenzae protein D. The nature of the carrier protein and the chemical methods for conjugation may influence immunogenicity of the vaccines [47,48,53,54].

4. What are adjuvants?

Adjuvants are substances that can enhance and modulate the immunogenicity of the antigen [55]. Adjuvants are usually not needed for live attenuated vaccines because these vaccines actively replicate and
self-enhance the immune response. However, they are frequently used for subunit vaccines because these vaccines contain fewer antigens and lack some of the intrinsic components present in whole pathogens that trigger the innate immune response, so that an effective downstream adaptive response is less likely to be achieved [55].

Due to their capacity to activate innate immune responses, adjuvants can broaden or extend responses and improve memory responses, therefore allowing to reduce the number of doses needed or the amount of antigen needed in each dose (dose sparing) [55]. These features can have important implications for improving global vaccine supply, as illustrated during the 2009–2010 influenza H1N1 pandemic. Adjuvants can also improve immune responses in populations having typically low responses (e.g. infants, older adults or immunocompromised individuals) [55].

For almost a century, aluminium salts (also known as alum) were the only adjuvant approved worldwide and they still remain the most widely used. Aluminium salts act primarily by directly activating innate immune cells leading to antibody production [56]. Although many adjuvants have been developed, only a few have reached licensure stage due to the necessity of finding the right match between the antigen and the adjuvant: MPL (a detoxified form of bacterial lipopolysaccharide), oil-in-water emulsions (MF-59), the AS combinations (AS01–04) and virosomes are currently approved for humans. Virosomes consist of spherical lipid layers assembled in vitro with viral proteins to resemble viral membranes [57]. They are currently used in influenza and hepatitis A vaccines.

Adjuvants may be combined to obtain the desired immune response, notably when cell-mediated immune responses are needed. For instance, AS03 is based on α-tocopherol (vitamin E) and squalene in an oil-in-water emulsion, whereas AS04 is based on MPL and aluminium salts [58]. AS03 is used in pandemic influenza vaccines (Pandemrix, Arepanrix, Adjupanrix, GSK) and AS04 is used in a hepatitis B virus vaccine (Fendrix, GSK) and a HPV vaccine (Cervarix, GSK).

5. Vaccines of the future

New vaccine designs and concepts are needed to improve existing vaccines or address unmet needs notably for pathogens with multiple serotypes (e.g. dengue, S. pneumoniae), antigenic hypervariability (e.g. human immunodeficiency virus) or an intracellular phase that are predominantly controlled by T-cell responses (e.g. tuberculosis, malaria) [11].

Vectored vaccines combine the advantages of live vaccines and subunit vaccines and are made from non-pathogenic infectious viruses expressing antigenic protein genes of a pathogen. Viral vectors derived from retroviruses, herpes simplex viruses, adenoviruses or poxviruses have been developed for vaccination against a wide array of pathogens, some of which have been evaluated in clinical trials over the last few years [59]. However, their use can be limited by pre-exposure to the viruses used as vectors, leading to high prevalence of pre-existing neutralizing antibodies against the vectors in humans, which can lead to early vaccine clearance and reduced immunogenicity [59].

Another promising approach is the development of nucleic acid-based vaccines. These vaccines work by inserting DNA or RNA that encode antigenic proteins into body cells (e.g. muscle or skin cells), which induces antigen presentation to the immune system triggering an immune response [60,61].

In addition to the conventional needle injections (intramuscular, subcutaneous and intradermal routes), alternative modes of vaccine delivery are also being developed [62]. These include administration through mucosal tissues (intranasal, oral or sublingual) or through the skin using microneedle or needle-free devices. Vaccination through these alternatives routes is likely to be easier and more comfortable for vaccine recipients and may improve vaccine acceptance and uptake, especially in those who fear needles [63,64]. Furthermore, they can induce both systemic and mucosal immune responses at the sites of pathogen entry [62]. However, despite much effort, vaccination through these alternative routes remains limited to a few vaccines (OPV, cholera vaccine, and rotavirus vaccines for the oral route and the live attenuated influenza vaccine for the nasal route).

6. Are vaccines safe and always effective?

Like all medicines, vaccines can have adverse events. However, because vaccines are given as preventive measures mostly to healthy individuals, especially infants and children, a highly positive benefit–risk profile is essential. Vaccine safety is evaluated in the preclinical and clinical phases of development but is also continuously monitored after licensure. Surveillance of vaccination programs and reporting by healthcare providers are essential to detecting rare or serious adverse events linked to vaccination, such as intussusception with the first rotavirus vaccine Rotashield (Wyeth) or narcolepsy with the adjuvanted H1N1 influenza vaccine Pandemrix (GSK) [23,65]. However, further research is needed to confirm what role Pandemrix
may have played in the development of narcolepsy among those affected. Indeed, narcolepsy is a complex disease whose causes are not yet fully understood but it may be associated with genetic and environmental factors, including infections [66].

Despite the recent successful developments in vaccine design, no vaccine provides an absolute or lifelong protection for all vaccinated individuals. In some cases, vaccines fail to induce a protective immune response. These vaccine failures are illustrated by breakthrough cases or are detected through serological testing of high-risk populations (e.g. hepatitis B in healthcare providers, rubella in pregnant women). For this reason, other available preventive measures should not be neglected, such as regular cervical screenings in women vaccinated against HPV [67].

7. Conclusions

Our improved understanding of the immune system and host-pathogen interactions has allowed transition from an empirical to a more rational vaccine design, but progress is still needed to address unmet needs and improve protection induced by current vaccines. Vaccines with a similar indication can rely on very different concepts with their own benefits and limitations. Understanding the basic concepts of vaccines and their recommendations for use is therefore crucial to understand their benefits and risks. Notably, the number of cases and deaths from infectious diseases prevented by direct and indirect effects of vaccination worldwide and the economic benefit to societies by cost of prevention should be acknowledged. Improving public knowledge about vaccines should support the appropriate expectations of vaccines, increase confidence in the science of vaccination, and can help reduce vaccine hesitancy.

Acknowledgements

The authors thank Drs. Lode Schuerman and Alberta Di Pasquale (GSK, Belgium) for their critical review of the manuscript and Sarah Brown (Fishawack, United Kingdom) for the figure. Writing assistance was provided by Dr. Julie Harriague (4Clinics, France), on behalf of GSK. Authors would like to thank Business & Decision Life Sciences platform for editorial assistance and manuscript coordination, on behalf of GSK. Carole Desiron coordinated manuscript development and editorial support.

Disclosure statement

VV, LRF and JK are employees of the GSK group of companies and hold shares in the GSK group of companies. GD is an employee of MSD and was an employee of the GSK group of companies. MS has received speaker honoraria from Merck, Amgen, Pfizer, NovoNordisk and Allergan outside of this submitted work and is an advisory board member for the GSK group of companies, Mithra, Pfizer and Amgen outside this submitted work.

Adjupanrix, Arepanrix, Bexsero, Boostrix, Cervarix, Engerix B, Fendrix, Flulaval, Havrix, Infanrix, Ixiaro, Mencevax, Menvveo, Mosquirix, Nimenrix, Pandemrix, Priorix, Priorix-Tetra, Rotarix, Synflorix, Shingrix and Tritanrix are trademarks owned by or licensed to the GSK group of companies. Gardasil, Gardasil 9, M-M-RVAXPRO, Pneumovax 23, ProQuad, Recombivax HB, RotaTeq, Vaqta and Zostavax are trademarks of MSD (Merck Sharp & Dohme) or Merck & Co, Inc. ACT-HIB, Adacel, Dengvaxia, GenHevac-B, Imojev, Menactra, Pneumo 23, Stamaril and Vaxigrip are trademarks of Sanofi Pasteur. Neisvac, Prevnar, Prevnar 13 and Trumena are trademarks of Pfizer. Fluenz and Flumist are trademarks of MedImmune, LLC. Flucelvax is a trademark of Seqirus. Rotavac is a trademark of Bharat Biotech. Dukoral is a trademark of Valneva Sweden AB.

Funding

This work was supported by GlaxoSmithKline Biologicals S.A., which paid for all costs associated with the development and the publishing of the present manuscript.

ORCID

Volker Vetter http://orcid.org/0000-0002-8414-1657
Leonard R. Friedland http://orcid.org/0000-0001-9588-2306

References

[1] Delany I, Rappuoli R, De Gregorio E. Vaccines for the 21st century. EMBO Mol Med. 2014;6:708–720.
[2] Tognotti E. The eradication of smallpox, a success story for modern medicine and public health: what lessons for the future? J Infect Dev Ctries. 2010;4:264–266.
[3] Siegrist C-A. Vaccine immunology. In: Plotkin SA, Orenstein WA, Offit PA, editors. Vaccines. 6th ed. Philadelphia, United States: Elsevier/Saunders; 2013. p. 14–32.
[4] Clem AS. Fundamentals of vaccine immunology. J Glob Infect Dis. 2011;3:73–78.
[5] Kim TH, Johnstone J, Loeb M. Vaccine herd effect. Scand J Infect Dis. 2011;43:683–689.
[6] Fine PE. Herd immunity: history, theory, practice. Epidemiol Rev. 1993;15:265–302.
[7] Orenstein W, Seib K. Mounting a good offense against measles. N Engl J Med. 2014;371:1661–1663.
[8] Swamy GK, Heine RP. Vaccinations for pregnant women. Obstet Gynecol. 2015;125:212–226.
[9] Paterson P, Meurice F, Stanberry LR, et al. Vaccine hesitancy and healthcare providers. Vaccine. 2016;34:6700–6706.
[10] Hajj Hussein I, Chams N, Chams S, et al. Vaccines through centuries: major cornerstones of global health. Front Public Health. 2015;3:269.
[11] Pulendran B, Ahmed R. Immunological mechanisms of vaccination. Nat Immunol. 2011;12:509–517.
or glycoconjugate vaccines. Expert Rev Vaccines. 2011;10:307–322.

[53] Kim JS, Laskowich ER, Arumugham RG, et al. Determination of saccharide content in pneumococcal polysaccharides and conjugate vaccines by GC-MSD. Anal Biochem. 2005;347:262–274.

[54] Poolman J, Frasch C, Nurkka A, et al. Impact of the conjugation method on the immunogenicity of Streptococcus pneumoniae serotype 19F polysaccharide in conjugate vaccines. Clin Vaccine Immunol. 2011;18:327–336.

[55] Pasquale AD, Preiss S, Silva FT, et al. Vaccine adjuvants: from 1920 to 2015 and beyond. Vaccines (Basel). 2015;3:320–343.

[56] Wen Y, Shi Y. Alum: an old dog with new tricks. Emerg Microbes Infect. 2016;5:e25.

[57] Moser C, Muller M, Kaeser MD, et al. Influenza virosomes as vaccine adjuvant and carrier system. Expert Rev Vaccines. 2013;12:779–791.

[58] Garçon N, Di Pasquale A. From discovery to licensure, the adjuvant system story. Hum Vaccin Immunother. 2017;13:19–33.

[59] Ramezanpour B, Haan I, Osterhaus A, et al. Vector-based genetically modified vaccines: exploiting Jenner’s legacy. Vaccine. 2016;34:6436–6448.

[60] Khan KH. DNA vaccines: roles against diseases. Germs. 2013;3:26–35.

[61] Deering RP, Kommareddy S, Ulmer JB, et al. Nucleic acid vaccines: prospects for non-viral delivery of mRNA vaccines. Expert Opin Drug Deliv. 2014;11:885–899.

[62] Stanberry LR, Strugnell R. Vaccines of the future. In: Garçon N, Stern PL, Cunningham AL, editors. Understanding modern vaccines: perspectives in vaccinology. Vol. 1. Amsterdam: Elsevier; 2011. p. 151–199.

[63] Shakya AK, Chowdhury MYE, Tao W, et al. Mucosal vaccine delivery: current state and a pediatric perspective. J Control Release. 2016;240:394–413.

[64] Arnou R, Frank M, Hagel T, et al. Willingness to vaccinate or get vaccinated with an intradermal seasonal influenza vaccine: a survey of general practitioners and the general public in France and Germany. Adv Ther. 2011;28:555–565.

[65] Sarkanen TO, Alakuijala APE, Dauvilliers YA, et al. Incidence of narcolepsy after H1N1 influenza and vaccinations: systematic review and meta-analysis. Sleep Med Rev. 2017 [Jun 20]. DOI:10.1016/j.smrv.2017.06.006

[66] Kornum BR, Knudsen S, Ollila HM, et al. Narcolepsy. Nat Rev Dis Primers. 2017;3:16100.

[67] Lee LY, Garland SM. Human papillomavirus vaccination: the population impact. F1000Res. 2017;6:866.

[68] Ellis RW, Rappuoli R, Ahmed S. Technologies for making new vaccines. In: Plotkin SA, Orenstein WA, Offit PA, editors. Vaccines. 6th ed. Philadelphia, United States: Elsevier/Saunders; 2013. p. 1182–1199.

[69] Hemming M, Vesikari T. Detection of rotateq vaccine-derived, double-reassortant rotavirus in a 7-year-old child with acute gastroenteritis. Pediatr Infect Dis J. 2014;33:655–656.