

TIMELINE

From empiricism to rational design: a personal perspective of the evolution of vaccine development

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Abstract | Vaccination, which is the most effective medical intervention that has ever been introduced, originated from the observation that individuals who survived a plague or smallpox would not get the disease twice. To mimic the protective effects of natural infection, Jenner — and later Pasteur — inoculated individuals with attenuated or killed disease-causing agents. This empirical approach inspired a century of vaccine development and the effective prophylaxis of many infectious diseases. From the 1980s, several waves of new technologies have enabled the development of novel vaccines that would not have been possible using the empirical approach. The technological revolution in the field of vaccination is now continuing, and it is delivering novel and safer vaccines. In this Timeline article, we provide our views on the transition from empiricism to rational vaccine design.

Vaccination is now entering the third century of its practice and it is the most important medical intervention that has ever been implemented. Recent studies report that, so far, vaccines have prevented more than 100 million cases of disease in the United States alone¹ and that every year they prevent 2.5 million deaths worldwide, which equates to preventing approximately 7,000 deaths each day². In spite of the age of the approach, vaccine science is currently leading the way in terms of the introduction of innovative technologies. Vaccination has the potential to become one of the most important tools for maintaining the well-being of present and future human generations.

In this Timeline article, we review the history of vaccination and we describe some of the scientific discoveries that were instrumental to the development of novel and safer vaccines that have now eliminated most of the infectious diseases that affect infants and children. We also provide our perspective on how new technologies will further advance the vaccine field. This article does not aim to provide exhaustive

coverage of all vaccine developments and we select, as examples, those stories that in our view best illustrate the general progression of approaches to vaccine design.

The empirical approach

The plague of Athens. For most of human history, immunity against communicable diseases has been achieved empirically, by exposure to natural infection or by vaccination with killed or attenuated microorganisms or toxins, without additional manipulations. The first written record of immunity from a contagious disease is contained in the description of the plague that affected Athens, Greece, in 430 BCE. In *The History of the Peloponnesian War*³, Thucydides reports that during the second year of the war between Athens and Sparta from 431 BCE to 404 BCE, a plague spread through Athens killing one-third of the population. The contagion was so devastating that people no longer cared about the law, women were liberated from their tight customs and the population lost faith in religion. Thucydides reports that sick and

dying individuals found most compassion in those who had recovered from disease because they knew from experience that the same person was never affected twice and therefore they had no fear for their own safety. Although today we still debate whether the plague of Athens was caused by typhoid fever, epidemic typhus, bubonic plague, smallpox or another infectious agent, the description of immunity following exposure to the disease is clear (FIG. 1). The practice of exposing individuals to natural infection to protect them from disease has been used until recently, with an example being the ‘measles parties’ that were a popular practice in the 1950s, whereby healthy children were invited to the house of a child who was diagnosed with measles to expose them to the infection.

Smallpox and variolation. The first report of an immunization being used to protect individuals from a contagious disease comes from China in approximately the tenth century^{4,5}. Here, pustules from individuals who were affected by mild smallpox were dried and blown into the noses of uninfected individuals or were inoculated into the scratched skin to deliberately infect them with a less severe form of the disease, so as to protect them from future exposure. This practice — known as ‘variolation’ from the Latin description of the disease as *varius* (meaning ‘spotted’) — was also used in Africa and it became increasingly popular in Europe during the eighteenth century, at a time when smallpox was causing half a million deaths each year⁶. There are detailed descriptions of the effects of variolation from this period⁷. Typically, after inoculation, children were affected by a severe illness that culminated 7–8 days post exposure in a high fever, senseless speech and shooting pain in the armpits. Usually, 3 weeks after inoculation, the inoculated parts cicatrized (healed to form scar tissue) and individuals were protected from future disease; however, sometimes variolation resulted in death. Many of the fears of vaccines that are still inherent in some individuals today may derive from this period, when variolation had unacceptable and severe side effects. Building on the rural knowledge that those

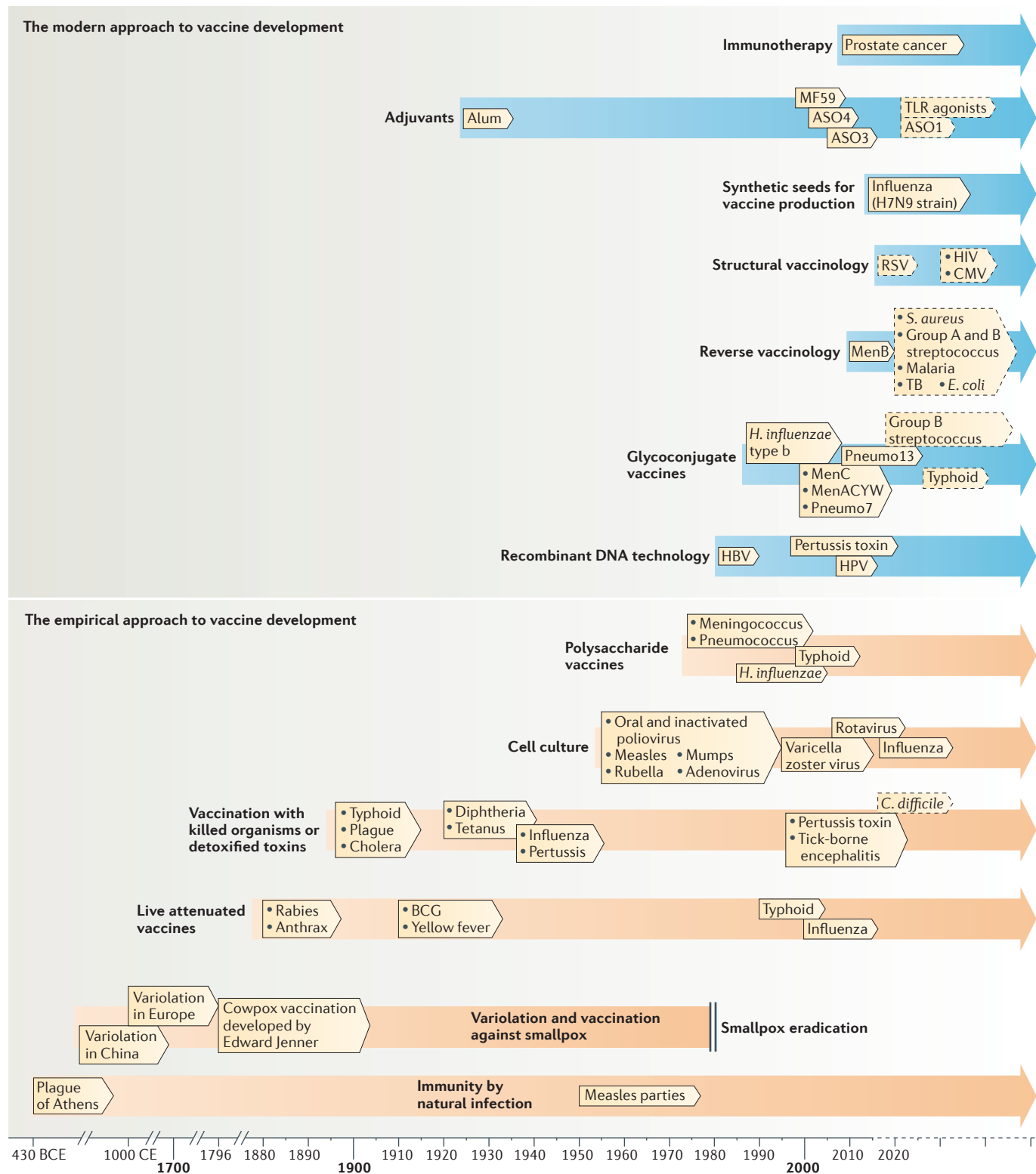


Figure 1 | A timeline of the history of vaccines showing the technologies that have enabled their development. Vaccine research can be divided into two main periods, with the first being the empirical approach, which was based on isolating, inactivating and injecting the microorganisms that cause disease. The second, modern approach began in the 1980s, when new technologies enabled advances in vaccine development that would not have been possible using the empirical approach. Closed boxes indicate licensed vaccines or vaccination practices that are

already used. Boxes with a dashed border indicate vaccines that are still in development. BCG, Bacille Calmette–Guérin; *C. difficile*, *Clostridium difficile*; CMV, cytomegalovirus; *E. coli*, *Escherichia coli*; *H. influenzae*, *Haemophilus influenzae*; HBV, hepatitis B virus; HPV, human papilloma virus; MenACYW, meningococcus serogroups A, C, Y and W; Pneumo7, 7-valent pneumococcus vaccine; Pneumo13, 13-valent pneumococcus vaccine; RSV, respiratory syncytial virus; *S. aureus*, *Staphylococcus aureus*; TB, tuberculosis; TLR, Toll-like receptor.

who contracted cowpox were immune from smallpox, in 1796 Edward Jenner used pustules from cows to achieve the same results as variolation but with much less severe side effects⁸. The discoveries of Jenner showed that a relatively easy source of 'attenuated' pustules was available and could protect from the devastating disease of smallpox. This is considered to be the official birth of vaccination. The procedure — named 'vaccination' from *vacca*, the Latin word for cow — became widespread and was used with few modifications until smallpox was fully eradicated in 1979 (REF. 9).

From microorganisms to vaccines. Jenner was not aware of the microbial origin of infections and had no concept of the mechanism of action of his vaccine. The scientific birth of vaccines came a century later when, following the discovery by Robert Koch and Louis Pasteur that infectious diseases are caused by microorganisms, Pasteur started to attenuate these microorganisms in the laboratory by drying, heating, exposing them to oxygen or passaging them in different animal hosts. The first microorganism to be attenuated was a bacterium that causes chicken cholera, which is now known as *Pasteurella multocida*¹⁰. The first human vaccine to be developed in this way contained a rabies virus that was grown in a rabbit spinal cord and attenuated by exposure to dry air. The vaccine was used in 1885 to successfully immunize Josef Meister, a boy who had been bitten by a rabid dog¹¹. Although this primitive rabies vaccine sometimes caused the death of the immunized individual, the procedure became very popular and people would come to Pasteur from all over Europe, Russia and the United States to be treated. An attenuated anthrax vaccine was produced at the same time. A few years later, following the discovery that diphtheria and tetanus are caused by bacterial toxins, Emil von Behring and Shibasaburo Kitasato found that the serum of animals that had been inoculated with these toxins could protect humans from disease^{12,13}. Building on the experience of Pasteur, Albert Calmette and Camille Guérin in Lille, France, passaged a *Mycobacterium bovis* strain 230 times to obtain an attenuated vaccine (Bacille Calmette–Guérin; BCG) against tuberculosis¹⁴. To this day, the BCG vaccine is the only clinically available vaccine against tuberculosis.

It thus became clear that to make a vaccine it was necessary to isolate, inactivate and inject the microorganisms that cause the disease or their toxins. The chemical

or physical inactivation of microorganisms was widely used in the early 1900s for the production of effective vaccines against typhoid fever, plague and cholera, and a few decades later against pertussis. Most of these vaccines, although effective, are no longer in use because of their high reactogenicity, with adverse effects including fever, pain and swelling at the injection site. In 1924, the chemical inactivation of toxins was described by both Gaston Ramon in Paris, France, and Alexander Glenny in London, UK, and this discovery led to the development of the diphtheria and tetanus toxoid vaccines that are still in use today^{15,16}.

Influenza vaccines were developed in the mid-1930s when it was found that the influenza virus could be grown in embryonated eggs — a procedure that is still used today to manufacture most influenza vaccines. A revolution in vaccine development came in 1949 when it became possible for the first time to grow viruses in cell culture. Starting with growing wild poliovirus *in vitro*, the new technology enabled the development of the inactivated¹⁷ and live attenuated¹⁸ polio vaccines during the 1950s. This was followed by the live attenuated vaccines against measles^{19,20}, mumps²¹ and rubella²² during the 1960s and, more recently, vaccines against varicella zoster virus²³, rotavirus²⁴ and influenza virus²⁵. All of these vaccines are still in use today.

During the 1960s, it became clear that individuals who had bactericidal antibodies specific for meningococcal polysaccharides were protected from meningococcal disease²⁶. Starting from the 1970s, this led to the development of purified polysaccharide vaccines against meningococcus serogroups A, C, Y and W^{27,28}, a 23-valent polysaccharide vaccine against pneumococcus^{28,29} and a vaccine against *Haemophilus influenzae* type b³⁰. These were the last vaccines to be developed using the empirical approach that was pioneered by Jenner and Pasteur, whereby to make a vaccine it was necessary to isolate, inactivate and inject the microorganism that causes disease or a component of the disease-causing microorganism. Starting from the late 1970s, new technologies became available that led the vaccine field away from the empirical approach and enabled the development of vaccines that would not have otherwise been possible.

Glycoconjugate vaccines

The capsular polysaccharide vaccines that were introduced in the 1970s against meningococcus, pneumococcus and *H. influenzae*, although effective in adults,

did not induce an immune response in infants. Polysaccharides are T cell-independent antigens that can stimulate existing memory B cell pools in pre-exposed adults but that are unable to induce B cell memory or the switch from IgM to IgG isotypes, or to stimulate the production of high-avidity antibodies during the primary immune response. As a consequence, polysaccharides are not immunogenic in infants, who are often those most in need of these vaccines.

In the 1970s, a group of scientists led by John Robbins at the US National Institutes of Health revisited a paper that was published by Oswald Avery and Walther Goebel in 1929 (REF. 31), which showed that when the pneumococcal polysaccharide was conjugated to a protein, it was able to induce antibodies (FIG. 2). Robbins and colleagues used the same technology to make a covalent conjugate of diphtheria toxoid linked to the capsular polysaccharide of *H. influenzae* type b, and in 1980 they described the first conjugate vaccine³². The conjugation technology heralded a major revolution in vaccinology. Within two decades, conjugate vaccines were licensed against *H. influenzae* type b, meningococcus serogroup C^{33,34} and seven serotypes of pneumococcus³⁵. In all cases, the vaccines induced high-affinity antibodies in infants, and they eliminated both the disease and the carriage of bacteria from the immunized population. During the past decade, the same technology has been used to develop vaccines against meningococcus serogroups A, C, Y and W^{36,37} and against six additional pneumococcus serotypes, making possible a 13-valent pneumococcal conjugate vaccine³⁸. Clinical studies have recently been carried out with conjugate vaccines against group B streptococcus³⁹ and typhoid fever^{40,41}.

Conjugate vaccines are the first example of vaccines that could not have been developed simply by growing microorganisms — as had been done since Pasteur — as the native form of the antigen must be transformed by linking it to a carrier protein in order to make the antigen immunogenic. We now know that conjugate vaccines work in naive individuals because the protein that is covalently linked to the polysaccharide is able to engage T cells and, therefore, the immune response to the polysaccharide becomes T cell dependent. Two mechanisms have been proposed to explain how conjugates engage T cells (FIG. 2b). In one model, the peptide derived from the carrier protein functions as a T cell epitope for a T cell that recognizes the protein itself. In the other

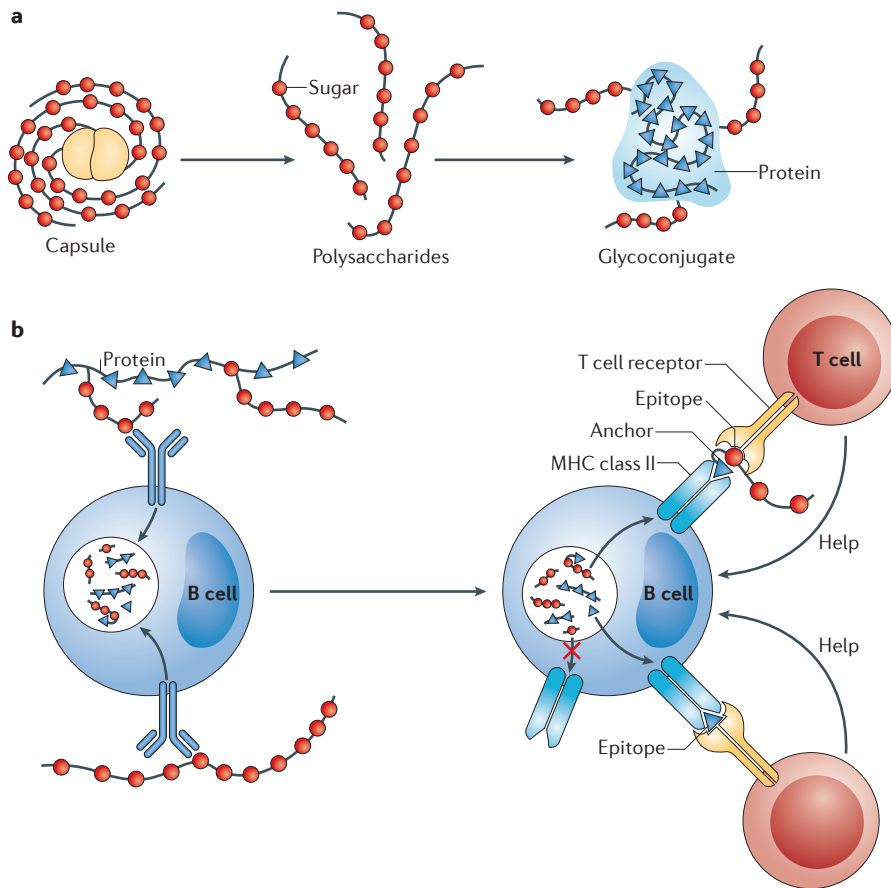


Figure 2 | Glycoconjugate vaccines. **a** | Polysaccharides that are present on the surface of capsulated bacteria are composed of many identical repeating units of simple sugars (represented by red circles). **b** | When purified, the polysaccharides are poorly immunogenic, as they are unable to enter the cavity of MHC molecules and therefore they fail to be presented to T cells. In conjugate vaccines, such as those against *Haemophilus influenzae* type b, pneumococcus, meningococcus and group B streptococcus, polysaccharides are covalently linked to proteins. The peptides that are derived from the proteins (represented by blue triangles) enter the cavity of the MHC molecule and engage the T cell receptor directly (bottom right) or function as an anchor for a sugar epitope (top right). Figure from REF. 42, Nature Publishing Group.

model, the peptide functions as a carrier to anchor the covalently linked sugar to the MHC molecule so that it can be recognized by a T cell that is specific for the carbohydrate⁴². To this day, we still know very little about the precise molecular mechanisms of the antigen processing, the nature of the T cell help or the immunological memory that is induced by glycoconjugate vaccines.

Recombinant DNA technology

In the mid-1970s, Maurice Hilleman wanted to make a vaccine against hepatitis B virus (HBV). However, HBV could not be cultivated in the laboratory and therefore he could not use the conventional approach of growing and inactivating the virus. Instead, Hilleman developed a vaccine by purifying and inactivating the virus-like particles (VLPs) that are found

in large quantities in the plasma of chronically infected individuals⁴³. The vaccine was effective but, in addition to obvious safety risks, a major limitation was the continuous need for infected individuals to provide the VLPs that were required to produce the vaccine.

During the same years, recombinant DNA technology became available, and Bill Rutter and Pablo Valenzuela at the University of California in Berkeley, USA, were able to clone the gene that encodes the surface antigen of HBV in a yeast system. From this, they could assemble the HBV antigen in VLPs that were antigenically identical to those that were purified from the plasma of infected patients⁴⁴. This new technology, initially commercialized by Merck and GlaxoSmithKline⁴⁵, made the unlimited production of the vaccine

possible by simply growing the yeast in fermentors. For the first time, a vaccine was produced without cultivating the micro-organism that causes the disease. A decade later, yeast and baculovirus were used to produce recombinant VLPs of other viruses that cannot be grown in laboratory cultures, including human papilloma virus 16 (HPV16) and HPV18 (REF. 46), which can cause cervical cancer. VLPs have recently become very popular in vaccine research and have been used to produce recombinant vaccines against many viruses. VLPs for influenza virus, respiratory syncytial virus (RSV), norovirus and parvovirus are currently being tested in early-phase clinical studies⁴⁷.

In addition to the production of viral antigens that could not be produced by the conventional approach of growing viruses in culture, recombinant DNA technology has also been used in the field of bacteriology to remove the toxicity from toxins. In the early 1980s, the inactivated whole-cell pertussis vaccine was highly criticized because of its real or purported side effects, such as fever and encephalopathy, and researchers were working to find a vaccine that was made from purified proteins (an acellular vaccine). Early work by Yuji Sato in Japan in the 1970s had shown that the culture supernatant of the bacterium contained two main proteins — pertussis toxin and filamentous haemagglutinin (HA)⁴⁸ — and Sato made a vaccine by chemical detoxification of the entire supernatant⁴⁹. In the Western world, scientists were developing vaccines by separately purifying the toxin and the HA, and adding a new antigen called pertactin and, in some cases, also fimbriae⁵⁰. In most of these vaccines, the pertussis toxin was detoxified by adding chemicals, using the same procedure described by Ramon and Glennly in 1924. In an alternative approach using recombinant DNA technology, the detoxification of pertussis toxin was achieved by cloning and sequencing the operon containing five of the genes that encode the toxin, and eliminating the toxicity by introducing two amino acid changes in the active site of the toxin^{51,52}. The mutagenized bacterium was able to produce unlimited quantities of a genetically inactivated pertussis toxin molecule. In ongoing clinical trials, this genetically inactivated toxin is tenfold more immunogenic than the chemically detoxified toxins, and it induces faster, stronger and longer-lasting immunity⁵³. In this case, recombinant DNA technology has been used to improve the quality and the safety of the antigen.

From genomes to vaccines

As described above, polysaccharide and conjugate vaccines have been successfully developed against meningococcus serogroups A, C, Y and W. However, the same technologies could not easily be applied to the development of a vaccine against meningococcus serogroup B, which causes approximately 50% of meningococcus cases globally. The capsular polysaccharide of this serogroup is composed of an ($\alpha 2 \rightarrow 8$)-linked polysialic acid, which is identical to the polysialic acid that is present in human glycoproteins, such as neural cell adhesion molecule 1 (NCAM1). The human immune system recognizes the meningococcal polysaccharide as a self antigen and, therefore, does not generate an antibody response against it. Some research groups have attempted to solve the problem by making modified conjugates in which the polysaccharide was chemically modified by introducing an *N*-propionyl group⁵⁴, and others have used purified proteins^{55,56}; however, none of these technologies has resulted in an effective vaccine. One approach that was partially successful was the development of outer-membrane vesicles (OMVs) that were obtained by detergent extraction of the bacteria⁵⁷. This procedure eliminated most of the toxic lipopolysaccharide from the bacterial outer membrane and extracted most of the loosely associated proteins but did not remove the well-anchored transmembrane proteins such as PorA, which induces protective immunity⁵⁷. OMV vaccines were successfully used in Cuba, Norway and, more recently, New Zealand; however, they were limited by the fact that they were only able to induce strain-specific protective immunity against the bacterium that was used to make the vaccine but not against bacteria expressing different PorA molecules. Studies by the Centers for Disease Control and Prevention (CDC) in the United States have indicated that at least 20 OMV vaccines would be required to make a combined vaccine that would be effective against 80% of meningococcus serogroup B strains⁵⁸.

In 1995, the publication of the first genome of a living organism (*H. influenzae*)⁵⁹ suggested that the solution to a meningococcus serogroup B vaccine could be found by sequencing the bacterial genome and selecting new antigens that had not been discovered by conventional technologies (FIG. 3). The process of genome-based antigen discovery is often described as 'reverse vaccinology'. An alliance between our vaccine development group at Chiron (now Novartis), the group of Richard Moxon (expert in meningococcal genetics) at the University of

Oxford, UK, and the laboratory of Craig Venter at The Institute for Genomic Research in San Diego, USA, started to work on decoding the genome of *Neisseria meningitidis* serogroup B in the search for novel antigens^{60,61}. Computer analysis identified novel candidate antigens that were then cloned and expressed in *Escherichia coli*. The novel proteins were used to immunize mice and the sera were tested for their ability to kill bacteria in the presence of complement. The new candidate antigens were prioritized for further investigation on the basis of bactericidal titre, sequence conservation between meningococcus serogroup B strains, the level of expression in different bacterial isolates and the absence of homology to human proteins. Finally, three of the candidate antigens were selected and combined with OMVs in the final vaccine formulation. In 2013, the first genome-derived vaccine to be developed by reverse vaccinology was licensed in Europe, Australia and Canada, and it has been used successfully in the United States for the mass vaccination of students at the universities of Princeton and Santa Barbara, where a meningococcus serogroup B outbreak had affected several students^{62,63}.

Although the path to licensure was slow for the meningococcus serogroup B vaccine, genome-based antigen discovery has been applied in one form or another to many other bacterial vaccines, including those against antibiotic-resistant bacteria, such as *Staphylococcus aureus*⁶⁴. In this case, the genome of *S. aureus* was used to

rapidly identify antigens in *E. coli* expression libraries that were recognized by serum antibodies from convalescent individuals. When sequencing of more than one genome per pathogen became possible, it was discovered that a single genome was often not sufficient to identify all protective antigens of a species and therefore multiple genomes were used to identify a universal vaccine against group B streptococcus⁶⁵. In the case of *E. coli*, genomes of pathogenic strains were compared with the genomes of commensal *E. coli* strains to identify only the pathogen-specific antigens⁶⁶. More recently, genome-based screens have also been used to identify antigens of *Chlamydia pneumoniae*⁶⁷ and *Mycobacterium tuberculosis*⁶⁸ that are recognized by T cells. Other examples of genome-based antigen discovery are those of *Porphyromonas gingivalis*⁶⁹, pneumococcus⁷⁰, group A streptococcus⁷¹ and many others, and in every case this technology has delivered novel antigens that can be used to formulate protective vaccines. As reverse vaccinology provides access to the entire antigen repertoire of bacteria and parasites, it is the most powerful antigen discovery tool that is currently available^{72,73}. Although the availability of genome sequencing represented huge progress in the discovery of novel vaccine antigens, this approach is not able to discover polysaccharide antigens or to solve the problems that are faced for vaccine design in the context of HIV or RSV, for which protective antigens are known but they are too variable or in the wrong conformation.

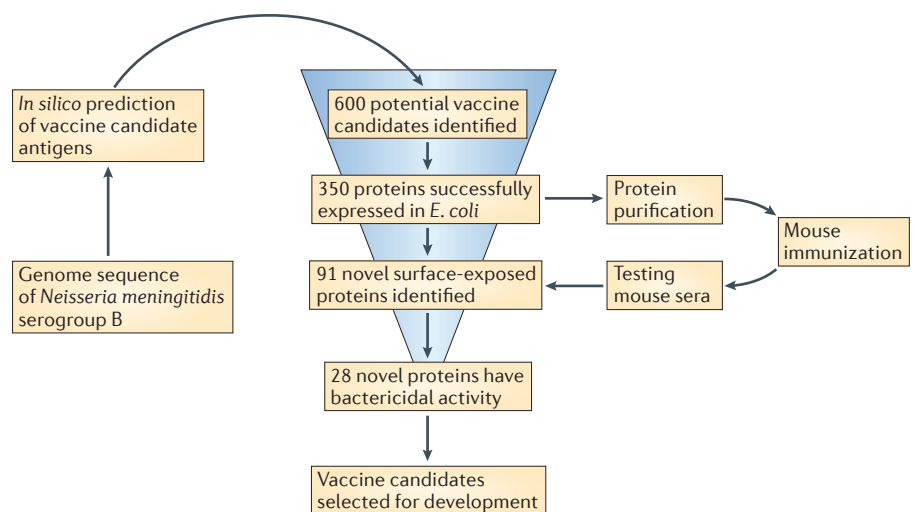


Figure 3 | Reverse vaccinology applied to meningococcus serogroup B. The genome sequence of meningococcus serogroup B is mined to predict genes encoding vaccine candidate antigens that are exposed on the surface of bacteria, that are antigenically conserved and that do not contain sequences that are similar to human proteins. The selected genes are expressed in *Escherichia coli* and then the proteins are purified and used to immunize mice. The sera of the mice are tested for their ability to kill bacteria in the presence of complement. The 'best' antigens are then selected for vaccine development.

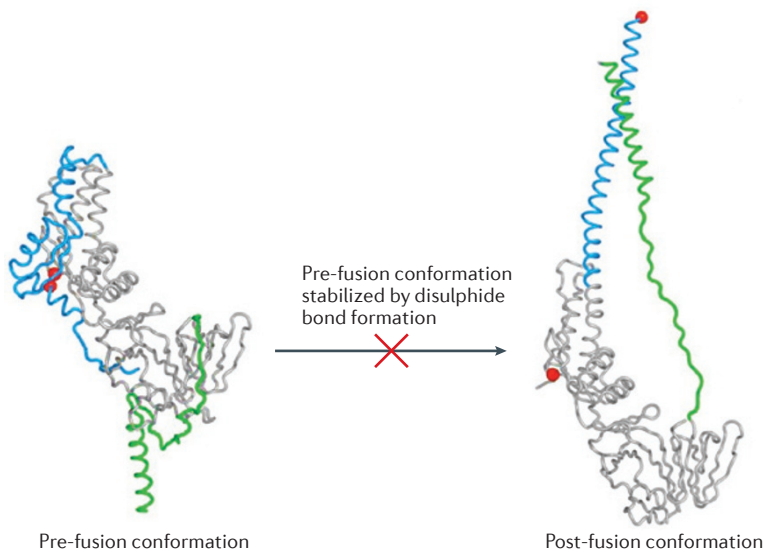


Figure 4 | Structural vaccinology for respiratory syncytial virus. The respiratory syncytial virus fusion (F) protein is unstable and flips easily from the pre-fusion conformation that is present on the surface of the virus (left) to the post-fusion conformation (right). The pre-fusion conformation contains more neutralizing epitopes and is preferred for vaccine development. Once the crystal structure of the pre-fusion and post-fusion conformations had been determined, it was possible to stabilize the pre-fusion conformation by introducing a cysteine at two amino acid residues (Cys155 and Cys290) that are closely spaced in the pre-fusion conformation but very distant in the post-fusion conformation (shown by red dots). The two cysteines in the pre-fusion conformation form a disulphide bridge and lock the protein in the pre-fusion state. From McLellan, J. S. Structure-based design of a fusion glycoprotein vaccine for respiratory syncytial virus. *Science* **342**, 592–598 (2013). Reprinted with permission from AAAS.

Structural vaccinology

During the past few years, progress in X-ray crystallography, nuclear magnetic resonance imaging and electron microscopy has substantially improved our ability to determine the three-dimensional structure of proteins. We can now obtain atomic-level information about key antigens and their epitopes, either alone or in complex with protective antibodies. This enables the use of structure-based design to modify antigens and make them ‘better’ immunogens⁷⁴. A few examples have already been described in which, on the basis of structural information, the sequence of an antigen has been modified to make it a better immunogen. In one case, the surface of factor H-binding protein of meningococcus was engineered to contain non-overlapping epitopes from three meningococcal antigenic variants, which resulted in a single molecule that could induce protective antibodies against all sequence variants⁷⁵. In a second example, the three-dimensional structure of the pre-fusion form of the fusion (F) protein of RSV enabled the rational design of a better immunogen. The F protein was locked in the naturally unstable pre-fusion conformation by introducing cysteine mutations at two amino

acids that are closely spaced in the pre-fusion form but that are very distant from each other in the post-fusion form of the same molecule⁷⁶ (FIG. 4). The resulting stable pre-fusion F protein induced higher neutralizing antibody titres than the existing post-fusion F protein. This development is likely to make it possible to develop the first safe and effective vaccine against RSV⁷⁷. These examples are just the beginning of an approach that is predicted to become routine in vaccinology and that might lead to the development of vaccines for diseases that remain difficult to target, such as HIV (BOX 1).

Synthetic seeds for influenza vaccines

In 2010, a research group led by Craig Venter successfully transplanted a synthetic genome of more than half a million bases into the cytoplasm of *Mycoplasma genitalium*⁷⁸. This event raised the possibility of using the *in vitro* synthesis of large portions of DNA and RNA for developing vaccines. The first vaccine to be developed using synthetic biology was tested in humans in 2013. On 24 April 2013, the Chinese CDC reported the discovery of H7N9 — a new and potentially pandemic avian influenza virus strain — and they made the sequence

of the HA and neuraminidase (NA) antigens from this virus available online. The next day, Venter’s laboratory synthesized synthetic DNAs that encoded the HA and NA antigens of the H7N9 strain, which were used to transfect cells, together with linear plasmids encoding the other six RNA segments of the influenza virus genome⁷⁹. A few days later, a new virus that was generated from the synthetic genes was isolated. This virus was used as a seed to manufacture a subunit vaccine in cell culture. The subunit vaccine was tested in a Phase I clinical study, where it was shown to induce protective levels of antibodies⁸⁰. This was the first human trial of a vaccine that was derived using synthetic biology. This technology provides a clear advantage in accelerating vaccine availability in the case of a pandemic (FIG. 5).

In parallel, the synthetic HA gene from the H7N9 strain of influenza virus was used in the self-amplifying mRNA (SAM) system⁸¹, which is an entirely synthetic RNA vaccine that is delivered by lipid nanoparticles. The vaccine was ready for animal immunization 8 days after the H7N9 virus was first reported, and mice had developed protective titres of HA-specific antibodies by 3 weeks after the second immunization, which equates to less than 40 days after the first report of the H7N9 virus⁸².

These examples are entirely disruptive of the traditional way of making influenza vaccines. Usually, influenza viruses are collected and shipped to qualified centres — such as the CDC in the United States or the National Biological Standardization Laboratories in the United Kingdom — where the viruses are co-cultured in eggs to make reassortants and then sent to vaccine manufacturers. The possibility of using genome sequences to derive vaccine seeds or totally synthetic vaccines — as has been pioneered for the H7N9 influenza virus — could become routine not only for influenza virus but also for many other infectious agents, and we think that it will be particularly useful in the case of emerging dangerous infectious agents, such as severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV).

For nearly two centuries — from Jenner in 1796 to the 1980s — vaccine innovation was driven by the discovery of new infectious agents or by the discovery of how to cultivate them in order to facilitate the large-scale production of killed or live attenuated vaccines. During the past 30 years, we have seen the development of

Box 1 | The challenge of vaccines for HIV, tuberculosis and malaria

HIV, tuberculosis and malaria — which are known as the ‘big three’ killer diseases — are major challenges for global health and for vaccine design. The approaches that are described in this article have not yet been successful in developing effective vaccines against these diseases; however, in the case of HIV and malaria, the proof of concept that protective vaccines can be developed has been achieved.

HIV

Since the beginning of the HIV epidemic, the virus has infected more than 75 million individuals. Approximately 35 million of those individuals are still alive, and every year there are approximately 2.3 million new cases of HIV and 1.6 million deaths⁹⁴. Antibody-inducing vaccines that use the gp120 recombinant envelope protein^{95,96} of HIV or T cell-inducing vaccines that use adenoviral vectors^{93,97} have failed to show any protection in efficacy trials. In 2009, a canarypox virus vector (ALVAC) encoding HIV antigens plus two booster injections of the gp120 subunit AIDSVAX vaccine (Genentech) showed 30% protection in a trial that was carried out in Thailand and provided the proof of principle that vaccine-mediated prevention of HIV is possible⁹⁸. Currently, new antigens and new adjuvants are being developed to improve the efficacy of a preventative HIV vaccine. The recent discovery of highly protective and broadly neutralizing antibodies⁹⁹ suggests that structural vaccinology approaches have the potential to deliver improved vaccines.

Tuberculosis

Mycobacterium tuberculosis infects one-third of the global population; 8.6 million new cases are reported each year and it causes 1.3 million deaths every year¹⁰⁰. The Bacille Calmette–Guérin (BCG) vaccine is a live attenuated bacterial vaccine that was developed in the 1920s and that is still used to vaccinate most children globally. Although the BCG vaccine protects infants from severe disease, it is not able to prevent infection in developing countries and, increasingly in urban centres of developed countries, the disease develops again during adolescence or adulthood and it can be highly lethal in HIV co-infected individuals. So far, it has been difficult to develop vaccines that perform better than BCG in animal models, which unfortunately only measure short-term protection. However, promising new vaccines based on engineered live attenuated bacterial strains, vectors and recombinant proteins that are combined with new adjuvants are currently being tested in a joint effort led by AERAS¹⁰¹.

Malaria

Malaria causes 200 million new cases and more than 600,000 deaths every year¹⁰². Recently, the RTS,S vaccine, which is based on a hepatitis B virus-like particle that expresses a portion of the malaria surface antigen, combined with the AS01 adjuvant, has been shown to prevent from 30% to 57% of infections in several efficacy studies in Africa⁸⁸. These studies show that malaria prevention by vaccination is possible, and while the RTS,S vaccine (GlaxoSmithKline) is being proposed for licensure, new improved vaccines are being developed using novel adjuvants, genome screening for new antigens, engineering of known antigens by structural vaccinology and prime–boost strategies using viral vectors (for further information, see the [PATH Malaria Vaccine Initiative](#) website). Interestingly, a vaccine composed of irradiated sporozoites that is delivered intravenously has shown 100% protection in adult volunteers¹⁰³. This result shows that although effective vaccines are possible, modern technologies for delivering irradiated sporozoites or multiple antigen vaccines that are delivered with vectors or novel adjuvants will be necessary.

to be introduced for human use and since then it has been used in hundreds of millions of individuals. MF59 greatly increases the immunogenicity of seasonal influenza vaccines, particularly in the elderly and in young children, and it is necessary for responses in all age groups in the case of a pandemic vaccine, where individuals have not been primed by natural influenza infection. In children aged from 6 to 72 months, the addition of MF59 increased the efficacy of seasonal trivalent influenza vaccine from 43% to 86%⁸⁴.

Other emulsions, such as AS03 (Glaxo-SmithKline)⁸⁵, have been used in the 2009 pandemic influenza vaccine and others, such as stable emulsion (SE), are being tested in preclinical and clinical studies⁸⁶. However, the field has undergone a marked change in recent years owing to the discovery of the signalling pathways of innate immunity, such as the signalling that is mediated by Toll-like receptors (TLRs). Thus, for the first time, the molecular targets of adjuvants were revealed and this made it possible to select natural agonists that are able to stimulate them. One such agonist is monophosphoryl lipid A (MPL), which targets TLR4 and was combined with alum to formulate AS04 (Glaxo-SmithKline), which is the adjuvant that is used in one of the licensed HPV vaccines⁸⁷. A TLR agonist has been combined in a liposome formulation in a malaria vaccine⁸⁸ and other TLR agonists, such as CpG oligonucleotides (TLR9 agonists), have been extensively tested in clinical trials as a component of vaccine formulations that are directed against various targets, including HBV⁸⁹. This is just the beginning of a new era in the adjuvant field. TLRs and other receptors — such as NOD-like receptors — can be used to screen for small molecules that can be optimized by medicinal chemistry to obtain synthetic ‘immunodrugs’ that are able to elicit the desired immune response. For example, a small molecule targeting TLR7 has been modified by attaching it to a linker with a phosphate group. This enables the small molecule to be adsorbed to alum and to deliver its adjuvant activity locally — that is, only to the antigen-presenting cells and the lymph nodes that are in contact with the vaccine antigens — thereby avoiding unnecessary stimulation of other tissues (E.D.G. and colleagues, unpublished observations; see Acknowledgements). The design of totally synthetic small-molecule adjuvants is an emerging field that is expected to develop in the near future.

technologies that have changed the nature of the vaccine itself and have enabled researchers to design vaccines for diseases that could not be tackled using conventional technologies. The technological revolution is continuing, not only providing new methods to make vaccine antigens but also new methods to deliver them using new formulations, and with new ways to improve their antigenicity and safety with novel adjuvants.

Adjuvants

In most cases, non-living vaccines require the help of immunostimulatory molecules known as adjuvants (from the Latin word

adiuvare meaning ‘to help’)⁸³. The first molecules to be used as adjuvants were phosphate or hydroxide salts of aluminium (known as alum). These were introduced in the 1920s for vaccines against diphtheria and tetanus toxoids, and since then they have been successfully used to formulate most of the non-living vaccines that have been administered to billions of infants and adults. Interestingly, no other adjuvant for human use was introduced for almost a century, mainly because the experimental adjuvants failed for manufacturability, stability or safety reasons in clinical trials. In 1997, an oil-in-water emulsion named MF59 (Novartis) was the second adjuvant

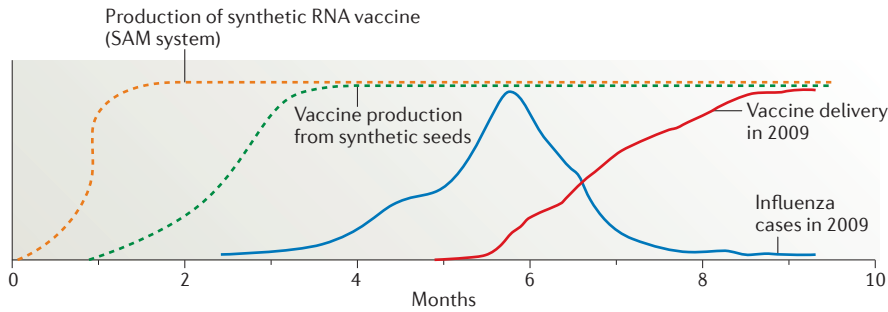


Figure 5 | **Synthetic biology for influenza vaccines.** Timeline of the 2009 influenza virus pandemic showing that by using the conventional technologies at that time, large quantities of vaccine became available only after the peak of the viral infection. The dashed lines indicate the hypothetical time course for vaccine production from synthetic seeds and the synthetic self-amplifying mRNA (SAM) system, which might help to produce large quantities of vaccine in the future before the peak of influenza infection. Figure adapted from REF. 111, with permission from AAAS. Data from REFS 112, 113; Douglas Jordan/Centers for Disease Prevention and Control.

Conclusions

During the past 30 years, new technologies have enabled advances in the development of vaccines that would not have previously been possible, and today, the most important diseases that used to kill or incapacitate millions of children are preventable by vaccination. The major infant vaccine that is still missing from clinical schedules is probably one against RSV. Recent progress in structural vaccinology indicates that RSV infection might soon be preventable through the development of a new generation vaccine. The vaccine field is currently

in the middle of a powerful technological revolution that is expected to deliver vaccines against those infectious agents that remain untargeted, such as antibiotic-resistant bacteria, HIV, malaria and tuberculosis (BOX 1). Reverse and structural vaccinology, combined with synthetic biology, are probably crucial in the development of vaccines for these difficult diseases, used alone or in prime-boost regimes with novel adjuvants. The emerging field of systems biology is going to increase our understanding of the molecular mechanisms of vaccine safety and immunity (BOX 2).

In addition, new technologies are expected to expand the use of vaccines to other fields. We expect to see vaccines that are able to prevent or cure metabolic diseases, neurodegenerative diseases and cancer by eliciting immunity against self antigens that have assumed pathological forms — such as β -amyloid in Alzheimer’s disease — or those that are overexpressed, as occurs in cancer. The licensure of the first therapeutic vaccine against prostate cancer, which consists of antigen-presenting cells that have been stimulated *in vitro* with the tumour antigen prostatic acid phosphatase⁹⁰, marks the beginning of a new era that is expected to deliver vaccines that are safe and able to prevent and cure disease of any type in all age groups. In this article, we have described the vaccinology approaches that, in our opinion, are the most promising for the development of novel vaccines or that have already contributed to licensed vaccines. Other approaches — such as anti-idiotypic vaccines⁹¹, DNA vaccines⁹² or pure T cell vaccines⁹³ — that have so far not shown efficacy in humans have not been described in detail. Some vector-based vaccines that are delivering promising results in clinical trials have only been mentioned in the context of some prime-boost studies and thus have not been discussed here.

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Box 2 | The application of systems biology to vaccine development

Vaccine safety and efficacy have classically been measured in large clinical trials that are designed to study whether vaccination induces side effects and protects against the natural acquisition of the disease. Some *in vitro* assays measuring levels of toxin-neutralizing or virus-neutralizing antibodies, the bactericidal or opsonic activity of serum and the numbers of antigen-specific T cells can, in some cases, be used as surrogate markers of efficacy. Recently, the ability to measure global changes in gene expression in the blood, combined with proteomics and deep sequencing of the B cell and T cell repertoires, has provided a new powerful tool to study the perturbations of the immune system that are induced by vaccination and adjuvant administration¹⁰⁴. These new technologies have been used to show that intramuscular administration of adjuvants induces marked changes in gene expression in mice and that different adjuvants induce a different gene expression profile¹⁰⁵. In humans, pioneering studies have shown that administration of the live attenuated viral vaccine against yellow fever induces a change in the profile of gene expression in peripheral blood mononuclear cells (PBMCs) and results in increased expression of genes that are involved in virus sensing and immunity^{106,107}. Interestingly, it was also shown that the expression of *GCN2* (also known as *EIF2AK4*) — a gene involved in sensing amino acid starvation that was not previously related to immunity — strongly correlated with the CD8⁺ T cell response to the yellow fever vaccine¹⁰⁸. This finding suggested a link between immunity and metabolism that had never been made by conventional technologies. More recently, systems biology studies of live and inactivated influenza vaccines, and of polysaccharide and conjugated meningococcal vaccines, have shown that each vaccine type induces a distinct gene expression signature in PBMCs¹⁰⁹, and this information has been used to build predictive models of post-vaccination antibody responses¹¹⁰. Systems biology holds promise to help dissect the molecular mechanisms of vaccine safety and efficacy, which, in the long term, should instruct the rational design of new-generation vaccines. In addition, systems biology could lead to the identification of novel biomarkers that can be used to predict vaccine efficacy, thereby avoiding large-scale efficacy trials.

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Competing interests statement

The authors declare [competing interests](#): see Web version for details.

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