nature milestones

Vaccines
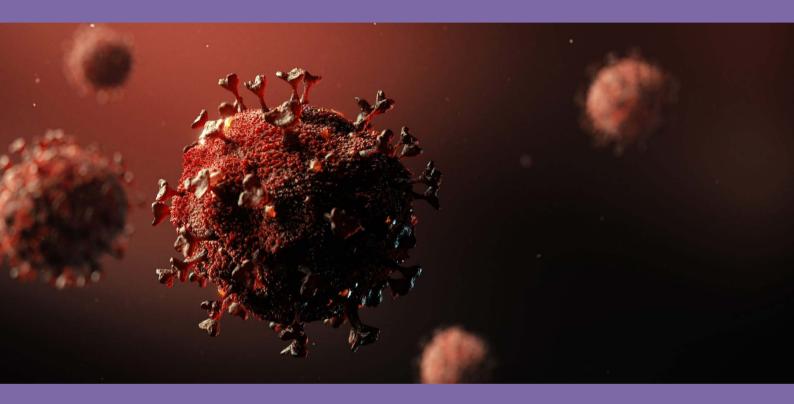
nature reviews
immunology

Collection: COVID-19 Watch

In this Collection, we bring you articles that highlight the latest research and insight into the immunology of SARS-CoV-2 and the associated disease COVID-19. They cover our emerging understanding of the immune response to this new coronavirus, prospects for vaccine development, immunopathology of COVID-19 and how it might be treated with immunomodulatory drugs.

Explore the Collection online
go.nature.com/COVID-collection

This *Nature Milestones in Vaccines* is published in the midst of the global SARS-CoV-2 pandemic, with hopes of a full return to pre-pandemic normalcy being pinned by many on the rapid development of a vaccine and implementation of a global vaccination programme. Indeed, more than 135 vaccine candidates are currently in pre-clinical and clinical development, using the whole range of available vaccine platforms. The extraordinary speed with which the scientific community has responded to this need for a new vaccine is the culmination of more than ten centuries of observation and study, starting with the practice of 'variolation' in India and the Ottoman Empire as far back as the 11th century (MILESTONE 1) on to the modern re-birth of smallpox vaccination by Edward Jenner in the 1790s (MILESTONE 2) and through to the latest developments in synthetic vaccines (MILESTONE 20) and individualized neoantigen vaccines for cancer (MILESTONE 21). These key steps in vaccine research, and many more in between, are summarized in the Timeline and covered in more detail in the Milestone articles. The topics for these Milestones were selected with the help of a panel of external expert advisors, and while we apologize in advance for any inadvertent omissions, we hope that they convey a sense of the true wonder of this field.

However, although vaccines have undoubtedly saved many millions of lives and are heralded as one of the greatest medical inventions of all time, this is also an era of increasing vaccine hesitancy, with surveys suggesting that a large percentage of the population are unsure whether they would opt to receive a COVID-19 vaccine even if one becomes available. The issue of public trust in vaccines (MILESTONE 19) has never been more important. Alarmingly, a recent publication in *Nature*, which we have chosen to include in the Collection of vaccine-related articles, showed that anti-vaccine groups are more effective than pro-vaccine groups at engaging with undecided groups on social media. This is no time for complacency; it is vaccination, not vaccines, that saves lives — a sentiment that is echoed in the included Review article by Peter Piot and colleagues. Also included in the Collection are shorter Comment and News & Views pieces that highlight topical issues concerning cancer vaccines, COVID-19 vaccines and new vaccine technologies.

The celebration of vaccine history presented in this *Nature Milestones in Vaccines* should not be taken for granted. Now, more than ever, our return to 'normal' may depend on it. Finally, we extend our sincere thanks to the advisors and acknowledge support from Emergent BioSolutions Inc. and Q² Solutions, and support of a grant from MSD. As always, Springer Nature takes complete responsibility for the editorial content.

Zoltan Fehervari, Senior Editor, *Nature Immunology*
Kirsty Minton, Senior Editor, *Nature Reviews Immunology*
João H. Duarte, Senior Editor, *Nature Biomedical Engineering*

MILESTONES IN VACCINES — TIMELINE

Year	Milestone
<1700	'Variolation' was practised in Asia as early as the 11th century (MILESTONE 1)
1798	Edward Jenner showed that cowpox infection could prevent smallpox (MILESTONE 2)
1881	Louis Pasteur discovered the process of bacterial attenuation (MILESTONE 3)
1890	Treatments using blood sera from recovered patients first described (MILESTONE 4)
1897	Standardized unit for diphtheria antitoxin
1901	Nobel Prize awarded for "the development of serum therapy"
1921	First in-human use of the BCG vaccine (MILESTONE 5)
1926	Discovery of aluminium salts as an adjuvant for vaccines (MILESTONE 6)
1937	Development of the attenuated 17D yellow fever vaccine strain by Max Theiler (MILESTONE 7)
1938	Effective tetanus vaccine developed, based on inactivated tetanus toxoid
1939	First effective vaccine for whooping cough (MILESTONE 8)
1945	First influenza vaccine approved
1949	First combination vaccine developed against diphtheria, tetanus and pertussis
	Poliovirus cultured in human cells for development of polio vaccine (MILESTONE 9)
1951	Nobel Prize awarded for discoveries concerning combating yellow fever
1954	Nobel Prize awarded for ability to culture poliovirus in various tissues
1955	Development of inactivated and live attenuated polio vaccines (MILESTONE 10)
1963	First measles vaccines licensed
1966	The Global Smallpox Eradication Programme launched by the WHO
1967	First mumps vaccine licensed
1969	The first live attenuated rubella vaccine licensed
1971	MMR vaccine licensed
1974	Expanded Programme on Immunization (EPI) founded
1979	Hepatitis B surface antigen cloned, leading to recombinant vaccine (MILESTONE 11)
1980	Smallpox declared eradicated
	Development of a new class of protein–polysaccharide vaccines (MILESTONE 12)
1984	Nonspecific effects of vaccines championed (MILESTONE 13)
1990	First experimental evidence for a protective HIV-1 vaccine
1991	Synthesis of human papillomavirus virus-like particles (VLPs) (MILESTONE 14)
1996	Albert Lasker Clinical Medical Research Award awarded for vaccines against meningitis
2000	Global Alliance for Vaccines and Immunization (Gavi) launched
	First example of 'reverse vaccinology' from genome to vaccine (MILESTONE 15)
2004	The quest for a safe vaccine against malaria considered a step closer (MILESTONE 16)
2006	First clinical trial of a dendritic cell-based cancer vaccine (MILESTONE 17)
2008	Systems biology approaches for examining vaccine responses in detail (MILESTONE 18)
2009	Vaccination against HIV-1 may reduce risk of infection in humans
2010	Narcolepsy linked to a pandemic influenza vaccine
	Public trust in vaccines at a low ebb (MILESTONE 19)
2011	Rinderpest declared eradicated
2013	Chemical synthesis of a vaccine in record time (MILESTONE 20)
2017	Coalition for Epidemic Preparedness Innovations (CEPI) launched
	Dengvaxia® controversy
	Individualized neoantigen vaccination for melanoma (MILESTONE 21)
2019	Ebola vaccine licensed

Credit: Chronicle / Alamy Stock Photo

▶ MILESTONE 1

The origins of vaccination

Edward Jenner (1749–1823), a physician from Gloucestershire in England, is widely regarded as the 'father of vaccination' (MILESTONE 2). However, the origins of vaccination lie further back in time and also further afield. In fact, at the time Jenner reported his famous story about inoculating young James Phipps with cowpox and then demonstrating immunity to smallpox, the procedure of 'variolation' (referred to then as 'inoculation'), by which pus is taken from a smallpox blister and introduced into a scratch in the skin of an uninfected person to confer protection, was already well established.

Variolation had been popularized in Europe by the writer and poet Lady Mary Wortley Montagu, best known for her 'letters from the Ottoman Empire'. As wife of the British ambassador to Turkey, she had first witnessed variolation in Constantinople in 1717, which she mentioned in her famous 'letter to a friend'. The following year, her son was variolated in Turkey, and her daughter received variolation in England in 1721. The procedure was initially met with much resistance — so much so that the first experimental variolation in England (including subsequent smallpox challenge) was carried out on condemned prisoners, who were promised freedom if they survived (they did). Nevertheless, the procedure was not without danger and subsequent prominent English variolators devised different techniques (often kept secret) to improve variolation, before it was replaced by the much safer cowpox 'vaccination' as described by Jenner.

But how did variolation emerge in the Ottoman Empire? It turns out that at the time of Lady Montagu's letter to her friend, variolation, or rather inoculation, was practised in a number of different places around the world. In 1714, Dr Emmanuel Timmonius, resident in Constantinople, had described the procedure of inoculation in a letter that was eventually published by the *Philosophical Transactions of the Royal Society* (London). He claimed that "the Circassians, Georgians, and other Asiatics" had introduced this practice "among the Turks and others at Constantinople". His letter triggered a reply from Cotton Maher, a minister in Boston, USA, who reported that his servant Onesimus had undergone the procedure as a child in what is now southern Liberia, Africa. Moreover, two Welsh doctors, Perrot Williams and Richard Wright, reported that inoculation was well known in Wales and had been practised there since at least 1600.

Patrick Russell, an English doctor living in Aleppo (then part of the Ottoman Empire), described his investigations into the origins of inoculation in a letter written in 1786. He had sought the help of historians and doctors, who agreed that the practice was very old but was completely missing from written records. Nevertheless, it appears that at the time, inoculation was practised independently in several parts of Europe, Africa and Asia. The use of the needle (and often pinpricks in a circular pattern) was a common feature, but some places had other techniques: for example, in Scotland, smallpox-contaminated wool (a 'pocky thread') was wrapped around a child's wrist, and in other places, smallpox scabs were placed into the hand of a child in order to confer protection. Despite the different techniques used, the procedure was referred to by the same name — 'buying the pocks' — which implies that inoculation may have had a single origin.

Two places in particular have been suggested as the original 'birthplace of inoculation': India and China. In China, written accounts of the practice of 'insufflation' (blowing smallpox material into the nose) date to the mid-1500s. However, there are claims that inoculation was invented around 1000 AD by a Taoist or Buddhist monk or nun and practised as a mixture of medicine, magic and spells, covered by a taboo, so it was never written down.

Meanwhile, in India, 18th century accounts of the practice of inoculation (using a needle) trace it back to Bengal, where it had apparently been used for many hundreds of years. There are also claims that inoculation had in fact been practised in India for thousands of years and is described in ancient Sanscrit texts, although this has been contested.

Given the similarities between inoculation as practised in India and in the Ottoman Empire, it may be more likely that variolation, as described by Lady Montagu, had its roots in India, and it may have emerged in China independently. However, given that the ancient accounts of inoculation in India are contested, it is also possible that the procedure was invented in the Ottoman Empire and spread along the trade routes to Africa and the Middle East to reach India.

Regardless of geographical origin, the story of inoculation eventually led to one of the greatest medical achievements of humankind: the eradication of smallpox in 1980. And of course, it inspired the development of vaccines for many more infectious diseases, turning this planet into a much safer place.

Alexandra Flemming,
Nature Reviews Immunology

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> On 14 May 1796, Jenner inoculated 8-year-old James Phipps with cowpox lesion material from milkmaid Sarah Nelms.

MILESTONE 2

Putting smallpox out to pasture

Although smallpox variolation dramatically reduced infection-induced fatality rates, it still carried significant risks, including the potential to trigger new smallpox outbreaks. In addition, it relied upon a constant supply of smallpox-infected individuals as a source of inoculation material. As variolation became more widely practised in the 18th century, an ostensibly simple observation started to gain more attention, with profound consequences for not only smallpox, but also many other infectious diseases.

In stark contrast to most individuals, dairy workers were generally protected from serious disease following smallpox exposure and lacked the permanent scars that often afflicted their non-dairy compatriots. Dairy farmers and milkmaids were in close and frequent proximity to cows, who sometimes developed pustules on their udders, symptoms of a zoonotic disease known as cowpox. In humans, cowpox generally manifested with pustules on the hands and arms, but was otherwise mild. Multiple reports of the protection afforded against smallpox by prior cowpox infection, in England and elsewhere, circulated in the 1760s. Although the relationship between the two diseases was unknown at the time, cowpox virus is a member of the *Orthopoxvirus* genus, which also includes variola virus, the causative agent of smallpox.

In 1774, Benjamin Jesty, an English farmer, leveraged this observation and inoculated his wife and two sons using pustule material from cowpox-infected cows. They remained healthy during subsequent smallpox epidemics, but he did not publish or further test his approach. Other reports of similar inoculations were made, but none appears to have received much attention and it is not clear whether Edward Jenner, an English physician, was aware of these reports prior to his own

more famous test. On 14 May 1796, Jenner inoculated 8-year-old James Phipps with cowpox lesion material from milkmaid Sarah Nelms. Phipps fell mildly ill, but recovered, and in July of that same year, Jenner formally tested the hypothesis that prior cowpox infection could prevent smallpox by variolating Phipps with smallpox lesion material. Phipps did not develop disease. Jenner's approach was eventually described as 'vaccination', a nod to its bovine heritage (*vacca* is the Latin word for cow). After his initial report of these findings was rejected by the Royal Society, Jenner self-published a longer monograph in 1798, documenting Phipps and an additional 22 cases that proved that cowpox, either through vaccination or natural infection, could protect against disease following smallpox variolation.

Jenner's results were met with some scepticism, but by 1800, vaccination had spread beyond England to other European countries and the United States. Since its initial iteration, the smallpox vaccine has itself evolved. In the 1800s, both cowpox and horsepox, which can also infect cows and humans, were used in parallel for immunizations. The exact virus in Jenner's original vaccine remains unknown. The modern smallpox vaccine contains vaccinia virus, which is related to, but genetically distinct from, cowpox virus. In spite of its popularization, the mechanisms

that contributed to the vaccine's protectiveness remained unclear until the 20th century. Studies in the 1970s suggested that pre-existing neutralizing titres were predictive of protection, pointing to a key role for antibodies in vaccine-elicited immune responses. In 2003, an analysis of individuals vaccinated 25–75 years earlier showed that 90% exhibited highly stable serum antibody titres and had vaccinia-specific T cells. Importantly, serum antibody titres correlated with neutralizing titres, and approximately 50% of those individuals still had antibody levels thought to be sufficient for protection against smallpox. Together with other studies, these data suggested that antiviral immunity following a single injection of the replicating vaccine was robust and potentially long-lived.

Jenner's initial arm-to-arm vaccination approach, which was more akin to the practice of variolation, remained common for some time. As vaccination spread globally, procedures for producing the vaccine were increasingly standardized, with serial passage of vaccine lymph in calves becoming the dominant approach after 1860. Variolation was formally outlawed as part of the Vaccination Act of 1840, and the Vaccination Act of 1853 made smallpox vaccination compulsory for all children born in England. Parents who chose not to vaccinate their children were subject to fines. This instigated the first anti-vaccination movement, which gained sufficient attention in Great Britain that a commission was appointed in 1896 to evaluate its concerns versus the benefits of vaccination. Although the commission concluded that smallpox vaccination was protective against disease, it also recommended against levying financial penalties, and a subsequent Vaccination Act in 1898 allowed parents to obtain a certificate of conscientious objection, a harbinger of things to come.

Following his first tests, Jenner continued to perform and promote smallpox vaccinations, presciently predicting that it could lead to the 'annihilation' of the disease, which had killed and afflicted so many. Less than two centuries after his first vaccination, Jenner was proved right and smallpox was declared eradicated by the World Health Organization in 1980.

Saheli Sadanand, Nature Medicine

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The first live attenuated vaccines

Awareness of Edward Jenner's pioneering studies of smallpox vaccination (MILESTONE 2) led Louis Pasteur (1822–1895) to propose that vaccines could be found for all virulent diseases.

Pasteur began to study chicken cholera in 1877 and by the following year had succeeded in culturing the causative organism, *Pasteurella multocida*. In 1879, Pasteur discovered by chance that cultures of this bacterium gradually lost their virulence over time. Before leaving to go on a holiday, Pasteur had instructed an assistant to inject the latest batch of chickens with fresh cultures of *P. multocida*. The assistant forgot to do this, however, and then himself went on holiday. On his return, Pasteur's assistant inoculated the chickens with the cultures, which by this time had been left in the laboratory for a month, stoppered only with a cotton-wool plug. The inoculated chickens developed mild symptoms but recovered fully.

Another scientist might have concluded that the cultures had (mostly) died, but Pasteur was intrigued. He injected the recovered chickens with freshly cultured cholera bacteria. When the birds remained healthy, Pasteur reasoned that exposure to oxygen had caused the loss of virulence. He found that sealed bacterial cultures maintained their virulence, whereas those exposed to air for differing periods of time before inoculation showed a predictable decline in virulence. He named this progressive loss of virulence 'attenuation', a term still in use today.

Pasteur, along with Charles Chamberland and Emile Roux, went on to develop a live attenuated vaccine for anthrax. Unlike cultures of the chicken cholera bacterium, *Bacillus anthracis* cultures exposed to air readily formed spores that remained highly virulent irrespective of culture duration; indeed, Pasteur reported that anthrax spores isolated from soil where animals that died of anthrax had been buried 12 years previously remained as virulent as fresh cultures. However, Pasteur discovered that anthrax cultures would grow readily at a temperature of 42–43 °C but were then unable to form spores. These non-sporulating cultures could be maintained at 42–43 °C for 4–6 weeks but exhibited a marked decline in virulence over this period when inoculated into animals.

Accordingly, in public experiments at Pouilly-le-Fort, France, conducted under a media spotlight reminiscent of that on today's COVID-19 treatment trials, 24 sheep, 1 goat and 6 cows were inoculated twice with Pasteur's anthrax vaccine, on 5 and 17 May 1881. A control group of 24 sheep, 1 goat and 4 cows remained unvaccinated. On 31 May all the animals were inoculated with freshly isolated anthrax bacilli, and the results were examined on 2 June. All vaccinated animals

Louis Pasteur holding rabbits, which were used to help develop the vaccine for rabies. Credit: World History Archive / Alamy Stock Photo

remained healthy. The unvaccinated sheep and goats had all died by the end of the day, and all the unvaccinated cows were showing anthrax symptoms. Chamberland's private laboratory notebooks, however, showed that the anthrax vaccine used in these public experiments had actually been attenuated by potassium dichromate, using a process similar to that developed by Pasteur's competitor, Jean Joseph Henri Toussaint.

In 1881, Victor Galtier (who had already demonstrated transmission of rabies from dogs to rabbits) reported that sheep injected with saliva from rabid dogs were protected from subsequent inoculations. These surprising observations piqued Pasteur's interest and he went on to develop the first live attenuated rabies vaccine.

Despite failing to culture the rabies-causing organism outside animal hosts or to view it under a microscope (because, unknown to Pasteur, rabies is caused by a virus rather than a bacterium), Pasteur discovered that the virulence of his rabies stocks, maintained by serial intracranial passage in dogs, decreased when the infected material was injected into different species. Starting with a highly virulent rabies strain serially passaged many times in rabbits, Pasteur air-dried sections of infected rabbit spinal cord to weaken the virus through oxygen exposure, as explained in Pasteur's 26 October 1885 report to the French Academy of Science. All 50 dogs vaccinated with this material by Pasteur were successfully protected from rabies infection, although we now understand attenuation to result from viral passage through dissimilar species, rather than air exposure.

Up to this point, however, Pasteur had no proof that his vaccines, a term coined by Pasteur to honour Jenner's work, would be effective in humans. Reluctantly — as Pasteur was not a licensed physician and could have been prosecuted for doing so — on 6 July 1885, Pasteur used his rabies vaccine, in the presence of two local doctors, to treat 9-year-old Joseph Meister, who had been severely bitten by a neighbour's rabid dog. Joseph Meister received a total of 13 inoculations over a period of 11 days, and survived in good health. Pasteur's reluctance might also be accounted for by posthumous analysis of his laboratory notebooks, which revealed that Pasteur had vaccinated two other individuals before Meister; one remained well but might not actually have been exposed, and the other developed rabies and died.

By the end of 1885, several more desperate rabies-exposed people had travelled to Pasteur's laboratory to be vaccinated. During 1886, Pasteur treated 350 people with his rabies vaccine, of whom only one developed rabies. The startling success of these vaccines led directly to the founding of the first Pasteur Institute in 1888.

Caroline Barranco,
Nature Reviews Cross-Journal Team

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MILESTONE 4

Serum power

Monoclonal antibody therapy is a cornerstone of modern care for non-communicable diseases, including cancer, autoimmune diseases and cardiovascular diseases. But long before the identification, isolation or cloning of antibodies, passive transfer of immune sera was used as a treatment for infectious disease — specifically tetanus and diphtheria — which were otherwise frequently lethal. Today still, antiserum from convalescent donors is being explored as a potential therapeutic intervention against viral infections, including those caused by ebolavirus and by pandemic SARS-CoV-2.

Yet the therapeutic potential of immune sera was first demonstrated more than 100 years ago in a series of animal experiments assessing immunity to the bacterial pathogens *Clostridium tetani* and *Corynebacterium diphtheriae* and

> "serum therapy is the foundation of … antibody-based immunotherapy"

their respective toxins. In 1890, Emil von Behring and Shibasaburo Kitasato reported that whole blood or cell-free serum from a rabbit previously injected with *C. tetani* could protect mice infected with a lethal dose of tetanus bacilli. Moreover, pre-treating tetanus toxin-containing bacterial filtrate with serum from an immunized rabbit blocked its lethality when it was subsequently injected into mice. Their landmark conclusions included that: cell-free components of the blood of a tetanus-immune rabbit had properties that could destroy the toxin; these properties were lacking in the blood of tetanus-naive animals; the tetanus-inactivating components were stably transferrable to *C. tetani*-infected animals via transfusion, in which they exerted a therapeutic effect.

One week after the report of these results, Behring published a related paper analysing immunity to *C. diphtheriae* in animals in which he demonstrated that transfer of antisera from immunized rats protected guinea pigs injected with diphtheria toxin. These findings

set the stage for what came to be called serum therapy — the transfer of sera from an immunized donor to a naive recipient to treat an infectious disease — and for which von Behring was awarded the very first Nobel Prize in Physiology or Medicine in 1901.

In 1894, the success of serum therapy in humans was first reported in children with diphtheria, a disease that accounted for 1% of all deaths of children under the age of 5 years at the time. When treatment with antisera was initiated early after diagnosis, nearly 100% of children recovered. Shortly thereafter, prevention of tetanus was achieved using horse antisera, which became a mainstay therapy of wounded soldiers during the First World War to prevent what had previously been a lethal disease. These successes with passive serum therapy also served to galvanize the research community to develop vaccine strategies that would actively elicit the protective antibodies generated naturally during infection.

The discovery that immunization with a bacterial pathogen or product could elicit a substance in serum with toxin-neutralizing properties — and which we now know to be antibodies — provided some of the first insights into humoral immunity that could account for the results of vaccination, as observed by Edward Jenner 100 years previously (MILESTONE 2). Elucidating the effects of antisera contributed to an understanding of hypersensitivity (observed owing to the use of animal antisera in humans) and the development of active vaccination for infectious disease. The demonstration of therapeutic efficacy using serum therapy is the foundation of today's antibody-based immunotherapy.

Alison Farrell,
Nature Medicine

Credit: SSPL/Getty Images

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MILESTONE 5

BCG: to face an ancient enemy

Credit: Alexey Kotelnikov / Alamy Stock Photo

Of all the infectious diseases that afflict humanity, tuberculosis is certainly one of the most ancient and implacable. Over the centuries this disease has gone by many names — 'phthisis', 'consumption', 'scrofula', 'the white plague'… and has killed more people than any other infectious disease in history — by some estimations in excess of a billion people in the past 200 years. However, it was not until 1882 that Robert Koch identified the bacterium *Mycobacterium tuberculosis* (Mtb) as the infectious agent to cause the most common form of the disease — pulmonary tuberculosis. The affliction that from antiquity had caused such untold misery at last had a face, and with it offered hope of a cure.

The final years of the 19th century were an exciting time for medicine: Louis Pasteur had successfully pioneered a number of attenuated vaccines (MILESTONE 3) and Shibasaburō Kitasato and Emil von Behring had demonstrated the antimicrobial properties of convalescent serum (MILESTONE 4). It was into this milieu that stepped the physician Albert Calmette and the veterinarian Camille Guérin. In 1894, Calmette had been appointed to be the first director of the Institut Pasteur in Lille, France, and along with Guérin — who was to become a lab head at the same institute, started

a close collaboration to produce an anti-tuberculosis vaccine. Their collaboration began in 1900 and would last until Calmette's death in 1933.

Calmette and Guérin's initial efforts focused on culturing a virulent bovine strain of Mtb in vitro with the hope that an attenuated version could be produced and thereby form the basis of a vaccine — much like Pasteur had managed with the cholera bacterium. However, the bacteria proved uncooperative and would readily form clumps, making them difficult to culture. A breakthrough came in 1906 when ox bile was included in the cultures to disperse the clumps and was found to weaken the bacteria. From 1908, Calmette and Guérin embarked upon a monumental subculturing effort to progressively attenuate their originally highly virulent bovine sample of Mtb. By 1919 and some 230 subcultures later, they finally had a live but highly attenuated strain of Mtb that was unable to cause disease in a wide variety of animals including guinea pigs, monkeys, calves and horses. This strain — now genetically vastly distant from its pathogenic ancestor — was christened Bacille Calmette–Guérin (BCG).

But human trials of BCG did not commence for some time, largely because of concerns that the bacteria might reacquire virulence

> “ BCG was not only very safe but might also be protective ”

following vaccination. BCG after all was a live organism so could they really be certain it was completely safe even if the animal data looked hopeful? Things changed in 1921 when they were approached by a physician working in Paris, Benjamin Weill-Hallé. He had as a patient a healthy infant whose mother had died of tuberculosis shortly after birth. The infant was to be raised by its grandmother who was also suffering from tuberculosis. The outcome in such cases was exceedingly grim so Weill-Hallé along with the paediatrician Raymond Turpin made the decision to orally vaccinate the infant with BCG. This was soon followed by a vaccination programme of similar at-risk newborn infants and appeared to show good protection of this vulnerable patient group. By 1927 a much larger programme involving thousands of infants demonstrated that BCG was not only very safe but might also be protective.

Nearly 100 years later, BCG is the most widely administered vaccine in the world and is on the WHO list of essential medicines. However, the global uptake of BCG is patchy, with a generally lower use in the developed world. This pattern partly reflects the relatively small tuberculosis risk and availability of antibiotics but also unresolved controversy over BCG's actual efficacy — which seems to be mainly useful in childhood against disseminated tuberculosis and tuberculous meningitis but relatively poor against the most common form of the disease in adults — pulmonary tuberculosis. However, BCG appears to have unexpected beneficial effects through the generalized stimulation of the immune system (MILESTONE 13), which can protect against pathogens other than its intended target Mtb and even some forms of cancer. It seems this most venerable of vaccines is still throwing up some surprises.

Zoltan Fehervari,
Nature Immunology

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Alum adjuvant discovery and potency

Vaccines have been lauded as one of the greatest scientific discoveries, having saved millions of lives from infectious diseases such as smallpox, and measles. Today, the ongoing COVID-19 pandemic is pinning much hope on a vaccine to save more lives. The success of such vaccines depends on their ability to elicit long-lasting immunity and protection from subsequent infections. This potency is highly dependent on adjuvants, which are incorporated in the vaccines to boost the immune response. The word adjuvant is derived from the latin word *adjuvare* meaning 'to aid'. Indeed, adjuvants have been used in vaccines to aid in their efficacy, especially for those using weak antigens.

Today, many vaccines are developed from components of pathogens. As such, adjuvants are required to provoke a strong immune response. The most widely used adjuvant is aluminium salt which was first used by the immunologist, Alexander T. Glenny, in 1926 at the Wellcome Physiological Research Laboratory in London.

In an attempt to purify and concentrate diphtheria toxoids (inactive toxin), Glenny and colleagues used potassium aluminium sulfate in the production of the vaccine.

Surprisingly, they found that vaccines developed using aluminium salt precipitation led to better antibody responses in guinea pigs than the soluble toxoids — the first demonstration of aluminium salt adjuvanticity. Glenny aptly stated in his article that "the antigenic value of the emulsion of precipitate appeared greater than that of the toxoid from which it came". Since then, numerous vaccines have been developed with 'alum' salts.

Adjuvants are also important in reducing the dose required for a vaccine. It is now known that combining adjuvants with recombinant proteins can significantly reduce the amount of antigen required to induce sufficient protective antibody production, ultimately reducing the dose administered. In addition, adjuvants can also broaden the immunity from vaccines by providing cross-clade immunity — immunity against different clades of pathogens with related origins. Importantly, adjuvants can also increase the magnitude of antibody responses.

The mechanism of action of adjuvants has been widely contested. In 1931, Glenny and colleagues initially proposed the 'depot theory', which suggests that through adsorption, alum facilitates slow release of

> " the antigenic value … of precipitate appeared greater than that of the toxoid from which it came "

the antigen into the injection site, thereby enhancing prolonged stimulation of the immune system. Glenny and colleagues found that alum nodules formed within a few hours in the injection site could be excised from an immunized guinea pig and subsequently implanted into a naive guinea pig, leading to successful immunization. However, work carried out over the past two decades has challenged this depot theory.

Recent work has suggested that the innate immune system plays a critical role. Following injection into the tissue, particulate adjuvants create a pro-inflammatory response by tissue-resident macrophages. This stimulates recruitment of innate immune cells such as neutrophils and subsequently dendritic cells. The dendritic cells play a crucial role in inducing an adaptive immune response.

In 1994, Polly Matzinger proposed the 'danger hypothesis' whereby localized tissue damage and cell death lead to release of danger signals such as uric acid, which ultimately trigger the innate and adaptive immune responses. Indeed, it has been shown that particulate alum salts lead to release of pro-inflammatory cytokines at the injection site. More recently, numerous studies have focused on the ability of alum adjuvants to activate inflammasomes, which are intracellular sensors that modulate inflammation in response to pathogens. Veit Hornung and colleagues showed that stress associated with phagocytosis of alum can trigger inflammasome activation.

There is a huge effort still required to fully understand the prevailing mechanism by which alum adjuvants regulate immunogenicity. However, it is clear that the pioneering work by Alexander Glenny on acquired immunity has been significant.

Amos Matsiko,
Nature Materials

Credit: S. Fenwick / Springer Nature Limited

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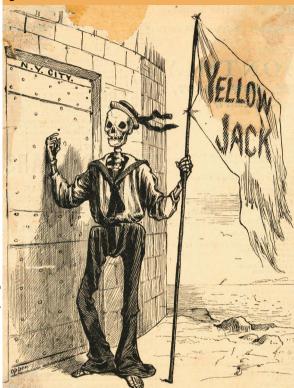**FURTHER READING** Oakley, C. L. Alexander Thomas Glenny, 1882–1965 (1966) | Reed, S. G., Orr, M. T. & Fox, C. B. Key roles of adjuvants in modern vaccines. *Nat. Med.* **19**, 1597–1608 (2013) | Matzinger, P. Tolerance, danger, and the extended family. *Annu. Rev. Immunol.* **12**, 991–1045 (1994) | Hornung, V. et al. Silica crystals and aluminum salts activate the NALP3 inflammasome through phagosomal destabilization. *Nat. Immunol.* **9**, 847–856 (2008)

MILESTONE 7

Developing the 17D yellow fever vaccine

Credit: Bettmann / Getty Images

> The 17D vaccine received licensing approval in 1938, with more than 850 million doses having been distributed since

By the end of the 19th century, the feared yellow fever (often known as 'yellow jack' owing to the yellow quarantine flag on infected ships) had reached South America, the USA and Europe. Caused by a zoonotic flavivirus spread by an infected female mosquito, mostly *Aedes aegypti*, the slave trade and global markets had helped to spread the disease around the world. Yellow fever is now endemic in large parts of sub-Saharan Africa and tropical South America, with the vast majority of cases occurring on the African subcontinent.

Yellow fever symptoms include chills, nausea, loss of appetite, headaches and muscle pain. In most people, these symptoms improve in around 5 days; however, for around 15% of cases, the fever returns with abdominal pain, jaundice and liver damage. Up to half of these individuals with severe disease will die.

Unsuccessful attempts to create a vaccine for yellow fever — including vaccines against a spirochaete or other bacteria — date back to the late 19th and early 20th centuries, before the causative agent of yellow fever had been identified. This is where South African virologist Max Theiler enters the story. Theiler started his work on yellow fever at the Harvard University School of Tropical Medicine in the USA. He and his colleagues confirmed that the disease was viral, and by 1928 they had shown that the same virus was responsible for both the African and South American pools of disease.

Researchers at the Rockefeller Foundation in the USA isolated the causative virus from the blood of a Ghanaian man called Asibi, and a team from the Institut Pasteur in Dakar, Senegal isolated the 'French strain' of the virus from a Lebanese man, Francoise Mayali. Once in the lab, these researchers found that serum from patients with yellow fever protected monkeys from infection but that killed virus was not effective at inducing immunity.

In 1930, Theiler moved to the Rockefeller Foundation in New York, where he worked to reduce the pathogenicity of the virus so that it could be used as a vaccine that triggered immunity but did not cause systemic damage. He showed that repeated passage in mouse brain cultures reduced the effect of the virus on most organs, but potentially increased its impact on the central nervous system, which could cause encephalitis. In 1931, after around 100 passages in mouse brain, the Rockefeller Institute tested a modified French strain as a vaccine, in combination with immune serum from recovered patients to reduce the risk of encephalitis. But the risk of neurotoxicity was still there and the large quantities of serum that were required made it hard to scale up its use. So, another approach was needed.

In a sequence of three publications in the *Journal of Experimental Medicine* in 1937, Max Theiler and Hugh Smith described the development of a live attenuated yellow fever vaccine strain using tissue from embryonated chicken eggs. The researchers focused on the Asibi strain, from which strain 17D was isolated after 176 passages initially in mouse embryonic tissue and monkey serum, and later in minced whole chick embryo, then in chick embryo from which the brain and spinal cord had been removed. 17D had lost neurotropism, viscerotropism and mosquito competence, but it still had the potential to trigger an immune response.

Ernest Goodpasture, a US pathologist and medic, should be given due credit for paving the way for this stage of the research. Working in the early 1930s, he and his fellow researchers were the first to reproducibly grow pure viruses in culture by infecting fertilized chicken eggs. Prior to this, viruses could only be studied in costly animal models or in tissue cultures that were prone to contamination by bacteria because antibiotics were not yet available.

The next step for Theiler and his team was to see whether a 17D vaccine prepared from infected whole chick embryos was safe and effective for human use without the addition of human serum, the bottleneck for Theiler's previous vaccine. In a study in rhesus monkeys, the vaccine triggered antibodies in all monkeys by 14 days and immunity to infection after a week. There were few adverse effects in the monkeys, so the researchers vaccinated four people who were already immune and eight people with no immunity. All developed antibody responses, and adverse effects were limited to mild fever, headache and backache. The positive results of this preliminary trial led to further larger studies.

The 17D vaccine received licensing approval in 1938, with more than 850 million doses having been distributed since. The vaccine is well tolerated, up to 100% effective and affordable, and it can provide lifelong protection with a single vaccination. Serious side effects are rare. In 1951, Theiler was awarded the Nobel Prize in Physiology or Medicine for "discoveries concerning yellow fever and how to combat it", the first and only time that the prize has been awarded for a vaccine.

Suzanne Elvidge

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Pertussis: a tale of two vaccines

Credit: Science Photo Library / Alamy Stock Photo

Pertussis, or 'whooping cough', has been identified since at least the Middle Ages as a disease that affects mostly infants and children, but can also affect teenagers and adults. The symptoms in the early stages of illness are similar to a cold, and then develop into a characteristic cough accompanied with gasping breaths ('whoops'). It is highly infectious; early 20th-century statistics claimed an average death rate of 10% in children.

A therapeutic vaccine of uncertain efficacy was developed shortly after the discovery of the causative agent, *Bordetella pertussis*, in 1906. However, it was not until 1939 that an effective preventive vaccine was designed. Thanks to improved cultivation methods, Pearl Kendrick, Grace Eldering and Loney Gordon developed a highly effective, whole-cell inactivated vaccine (wP). In the late 1940s the pertussis vaccine was combined with diphtheria and tetanus toxoids to become DTP and was widely adopted shortly afterwards.

By the early 1980s, the introduction of pertussis vaccines was associated with a large decline in cases, with incidence of whooping cough in the USA decreasing 150-fold. Unfortunately, as the risks from whooping cough decreased markedly, attention shifted from the risk of disease to fear of vaccine side effects.

> " By the early 1980s, the introduction of pertussis vaccines was associated with a large decline in cases "

The whole-cell pertussis vaccine causes rare but significant side effects. In addition to the usual local inflammatory effects and fever associated with many vaccines, whole-cell pertussis vaccines sometimes trigger prolonged crying and febrile convulsions and, very rarely, hypotonic–hyporesponsive episodes. Claims of causal links to various neurological issues were also made. However, those neurological side effects were observed in very small numbers of children and were later demonstrated to be unrelated to the vaccine, but the reputational damage was done.

The whole-cell pertussis vaccine was blamed for causing various intellectual and physical disabilities, including in a TV documentary. This precipitated the formation of 'Dissatisfied Parents Together', which would eventually become the National Vaccine Information Center, and remains to this day a major source of disinformation about vaccines in the USA.

Thousands of parents refused to vaccinate their children, and a flood of personal injury lawsuits forced many companies to stop producing vaccines. The US Congress passed the National Childhood Vaccine Injury Act, to protect vaccine manufacturers, which ironically is now used by vaccine deniers as 'proof' of the dangerous nature of vaccines. Three countries, Sweden, the UK and Japan, interrupted or decreased pertussis vaccination.

Meanwhile, in response to concern about the side effects, Yuji Sato was working on an acellular pertussis vaccine. There had always been resistance to the whole-cell vaccine in Japan, and, persuaded that the side effects were caused by products such as lipopolysaccharide and endotoxins present in the vaccine, he set out to create a less reactogenic vaccine. By 1974 his team had succeeded in producing an effective pertussis vaccine containing mainly just two antigens: pertussis toxin (PT) and filamentous haemagglutinin (FHA). This acellular pertussis vaccine (aP) was shown to be effective, albeit less so than the whole-cell vaccine, and in 1981 was approved for use in Japan. Other countries adopted similar methods of producing acellular vaccines, usually with added pertactin and type 2 and type 3 fimbriae for better effectivity, and combined it to create the diphtheria, tetanus, acellular pertussis vaccine (DTaP). By the late 1990s, most high-income countries had switched to DTaP, although the cheaper DTwP remains the vaccine of choice in low- and middle-income countries.

This could be the end of the story, except that in countries using DTaP there is now a resurgence in cases of whooping cough, with the characteristic peak every 2–5 years that was observed in the pre-vaccine era. There are a number of hypotheses as to why this previously well-controlled disease is now making a resurgence. It has been suggested that the less-effective long-term protection (waning after 5–10 years) of DTaP has allowed the epidemic cycles to re-establish themselves. This is partly challenged by more recent studies that allege a long-lived, if imperfect, protection afforded by DTaP. Understanding of the transmission and contact networks is incomplete, and studies looking at vaccine evasion in *B. pertussis* are not conclusive.
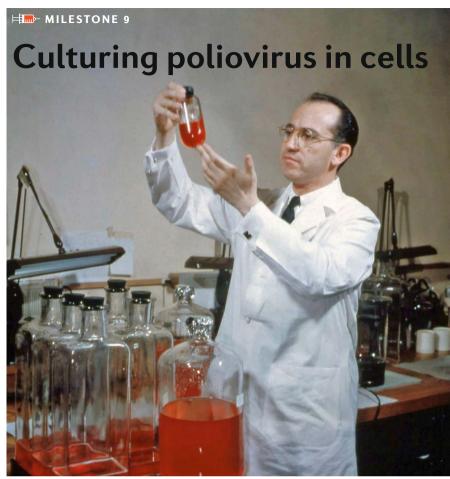
In the meantime, several countries are experimenting with new vaccination schedules and vaccination of pregnant women, and the search for a better vaccine continues.

Nicolas Fanget, *npj Vaccines*

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Culturing poliovirus in cells

Jonas Salk (1914–1995), who developed one of the first polio vaccines. Credit: Pictorial Press Ltd / Alamy Stock Photo.

The disease poliomyelitis (polio) is suspected to have been around for centuries and was initially defined as an infantile spinal paralysis. By the start of the 20th century, there were severe and frequent polio outbreaks in both the USA and Europe. In 1908, Karl Landsteiner and Irwin Popper demonstrated that polio was spread by a virus, by injecting two Old World Monkeys (*Cynocephalus hamadryas* and *Macacus rhesus*) with a suspension of spinal cord from a polio patient. The suspension was bacteriologically sterile; nevertheless, following inoculation the monkeys exhibited lesions in the spinal cord similar to those seen in humans with poliomyelitis. Additionally, the rhesus monkey developed paralysis of both legs.

Having been diagnosed with polio in 1921, President Franklin D. Roosevelt founded the National Foundation for Infantile Paralysis to combat polio in 1938. This provided funding to John R. Paul and James D. Trask, who found poliovirus in the faeces of both patients and their healthy contacts. By testing sewage water in New York it was later estimated that there was a ratio of 100 subclinical infections for each paralytic case, indicating widespread infection. Widespread vaccination therefore

seemed an appropriate strategy to tackle the spread of the disease.

Researchers were keen to find a method to culture poliovirus to facilitate development of a vaccine. Chicken eggs had been used to grow other viruses (MILESTONE 7); however, attempts to grow polio in chicken eggs were unsuccessful. Albert B. Sabin and Peter K. Olitsky grew a monkey-adapted strain of poliovirus (the MV strain) in fragments of human embryonic brain. However, there were concerns that such a culture system would not be suitable for growing large volumes of poliovirus for use as a vaccine. The major pathological features of patients with polio were of central nervous system origin and there was concern vaccine recipients could be at risk of central nervous system damage. Thomas H. Weller and John F. Enders had previously cultured mumps virus in non-neural tissue growing in test-tubes by suspending fragments of chick amniotic membrane in a salt solution containing components of ox serum. The amount of haemagglutinin, which is produced by the virus, could be measured in the culture fluid following inoculation of measles virus, providing a method to monitor

production of virus. They found that by replacing part of the medium regularly they could maintain viability of the cells whilst growing and harvesting virus. Funded by the National Foundation for Infantile Paralysis, they joined forces with Frederick C. Robbins to attempt to grow the human Lansing strain of human poliomyelitis virus in skin, muscle and connective tissue removed from human fetal arms and legs. In the *Science* paper in which their results were published, they reported maintenance of these cultures for 67 days. Over 52 days of culture they calculated at least 1,016 more virus particles were obtained than had been initially inoculated into the culture.

In addition, intracerebral inoculation of fluid from the cultures produced paralysis in mice and two rhesus monkeys. Microscopic examination of the spinal cords of the monkeys revealed lesions similar to those seen in humans with poliomyelitis. Earlier epidemiological studies had suggested the human intestine was also capable of producing large amounts of virus, excreted in the faeces. This was confirmed by production of large quantities of virus in cultures of fetal intestinal tissue that also induced polio-like symptoms in mice. The authors had succeeded in their aim to culture poliovirus in cells other than neurons. They also noticed that the morphology of cells grown in the presence of virus differed from their control cultures and concluded that the virus could be reducing the viability of the cells.

Long-term culture in different human tissues and in different culture conditions provided a starting point for the development of differing, attenuated strains of virus. It also enabled production of large quantities of virus, paving the way for Salk to produce a successful polio vaccine for human use (MILESTONE 10). The importance of the methodological advance of Enders, Weller and Robbins was recognized by the award of the 1954 Nobel Prize in Physiology or Medicine. Also following the successful introduction of polio vaccination, the funding body that funded their research, the National Foundation for Infantile Paralysis, was able to change its name to the March of Dimes and continues to this day to fund research to improve the health of babies.

Katharine Barnes, *Nature Protocols*
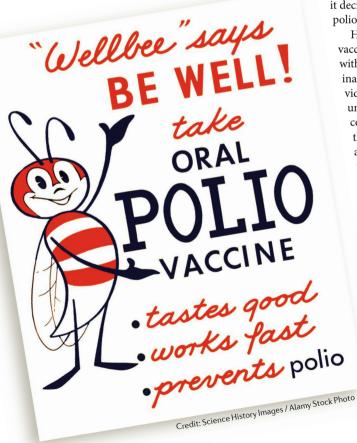
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Two polio vaccines for defeating a paralysing scourge

Poliomyelitis is caused by an enterovirus that in rare cases invades the nervous system and damages motor neurons, causing permanent disability, paralysis or death. Today, the disease has been eradicated from all but a handful of countries, thanks to two types of polio vaccine developed in the 1950s: an injected vaccine containing inactivated virus, originally developed by researchers led by Jonas Salk, and an oral vaccine containing live attenuated virus, originally developed by Albert Sabin and colleagues.

Researchers had been attempting to develop a polio vaccine since the 1910s, but early efforts were either ineffective or too risky. A key milestone occurred in 1949, when Thomas Weller, John Enders, and Frederick Robbins demonstrated that poliovirus

> **Between 1955 (…) and 1962, it decreased the incidence of poliomyelitis by about 95%**

could be grown in the laboratory using skin and muscle tissues from human embryos (MILESTONE 9). This meant that the virus no longer needed to be grown in live monkeys, facilitating its production in the necessary quantities for vaccine testing and production. The three researchers shared the 1954 Nobel Prize in Physiology or Medicine.

Jonas Salk developed his inactivated polio vaccine by growing the virus in monkey kidney cells, then killing the virus with formalin. A placebo-controlled trial in 1954 involving 1.6 million children in Canada, Finland and the United States showed that the killed poliovirus vaccine was safe and effective at preventing infection with wild poliovirus. Between 1955, when Salk's vaccine went into widespread use in the United States, and 1962, it decreased the incidence of poliomyelitis by about 95%.

However, antibody levels in vaccinated individuals decreased within a few years, so whether the inactivated vaccine would provide permanent protection was unclear. In addition, poliovirus could still multiply harmlessly in the guts of vaccinated individuals, so it was thought that Salk's vaccine would not fully interrupt the circulation of wild poliovirus in the population.

Meanwhile, researchers learned in the 1940s that passaging poliovirus repeatedly through rodents and then through cell culture resulted in strains that were less virulent. These observations laid the groundwork for a live attenuated poliovirus

vaccine, but this was a complicated endeavour: a weakened strain had to be found that could not enter the nervous system but that could still multiply in gut tissues and trigger the production of antibodies. The weakened strain then had to be purified and produced in large quantities without regaining neurovirulence. Sabin's discovery that chimpanzees are the best animal model species to test gut infectivity of attenuated strains, while cynomologous and rhesus monkeys are the best to test nervous system infectivity, helped guide these investigations.

Multiple researchers worked on the live attenuated polio vaccine effort throughout the 1950s, including groups led by Hilary Koprowski, Herald Cox and Sabin. By the time Sabin's strains were chosen as the safest in 1959, millions of doses of various experimental vaccines had been administered in studies around the world. The first nationwide mass vaccination campaign with Sabin's vaccine took place in Cuba in 1962, followed by other countries throughout the 1960s.

It soon became clear that the live vaccine occasionally caused poliomyelitis in vaccinated individuals, their contacts or members of the community. This occurred when random mutations accrued during replication of the vaccine virus in the intestine led to the regaining of neurovirulence. Thus, as countries brought wild poliovirus under good control, many switched to improved versions of the inactivated polio vaccine.

Countries where polio remains endemic continue to use the live oral polio vaccine because of its convenience and superior ability to induce mucosal immunity in the gut. Today polio continues to circulate in three countries: Afghanistan, Nigeria and Pakistan. Poor sanitation, lack of health-care infrastructure and opposition to vaccination campaigns by militant organizations have hampered the efforts to wipe out the virus for good.

Sarah DeWeerdt

Credit: Science History Images / Alamy Stock Photo

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A transmission electron micrograph showing the ultrastructural morphology of Dane particles — spherical HBV virions of ~42 nm in diameter. Credit: CDC.

MILESTONE 11

First recombinant DNA vaccine for HBV

In 1986, the Recombivax HB vaccine for hepatitis B was approved for human use in several countries, the culmination of research started by William Rutter, Pablo Valenzuela and colleagues in 1979 on the cloning of hepatitis B virus (HBV) antigens. It was the first vaccine to be produced using recombinant DNA technology and although it was only the third recombinant product to be approved for clinical use, it was also the most complex in forming nanoparticles that resemble patient-derived virus particles in both structure and immunogenicity.

Infection with HBV leads to the production of intact spherical virions of ~42 nm in diameter, also known as Dane particles, as well as the overproduction of 22 nm particles consisting exclusively of hepatitis B surface antigen (HBsAg). HBsAg is encoded by gene S, which contains three in-frame start codons that enable production of HBsAg proteins of three lengths (small, middle and large). The large HBsAg protein is the most abundant form found on the surface of infectious viral particles and is thought to have a crucial role in the binding of HBV to hepatocytes.

HBsAg was first identified in 1965 by Baruch Blumberg as an antigen found in the blood of an Aboriginal Australian and it was later shown by Blumberg and others to be associated with HBV infection and to be part of the virus itself. For his discoveries "concerning new mechanisms for the origin and dissemination of infectious diseases", Blumberg was recipient of a joint Nobel Prize with Carleton Gajdusek in 1976.

> **"** The ability to produce immunogenic HBsAg in genome-free virus-like particles... allowed for the large-scale production of HBV vaccines unable to infect host cells **"**

Given the failure since its discovery to cultivate HBV in vitro, the first commercial HBV vaccine (Heptavax; licensed in 1981) was based on inactivated virus collected from the plasma of HBV-infected donors. However, plasma products at the time had been associated with HIV-1 and HCV transmission and vaccine supply was limited by the availability of chronic HBV carriers. Therefore, the use of recombinant DNA technology was an attractive option for development of a vaccine that solved both of these problems. Targeting HBsAg was also attractive, given that it was encoded by a single gene and thought to be closely involved in interactions with host cells.

In 1979, William Rutter, who had been involved in research on recombinant insulin and growth hormone, and colleagues, including Pablo Valenzuela at the University of California, San Francisco, successfully cloned HBsAg into *Escherichia coli* expression vectors, demonstrating the possibility of using recombinant HBsAg as an HBV vaccine. Using Dane particles isolated from human serum by one of their funders, Merck Sharpe and Dohme, the researchers synthesized double-stranded viral DNA and carried out restriction mapping and Maxam–Gilbert DNA sequencing to assemble the viral genome. Based on the 19 amino acids that had previously been identified at the amino terminus of HBsAg, they located the 892 bp genomic region encompassing the S gene. It corresponded to a single protein sequence, potentially forming a globular protein. They also

identified three potential glycosylation sites that could account for the two sizes of polypeptide that can be removed from the viral surface coat by detergents.

A few years later, in 1982, the same group, together with colleagues from the University of Washington, cloned HBsAg into yeast expression vectors. They used a plasmid that placed the coding sequence under the control of a constitutive yeast promoter, which enabled a high level of HBsAg to be made, as verified by immunoassays. Remarkably, sedimentation as well as electron microscopy experiments showed that 22 nm particles were the predominant form of HBsAg secreted by the transformed yeast cells, similar to virus-infected human cells. Also like the 22 nm HBsAg particles from human cells, which had been shown previously to be ~1,000-fold more immunogenic than the unassembled HBsAg protein, the yeast-generated particles were recognized by the HBsAg-specific antibodies known at the time.

The ability to produce immunogenic HBsAg in genome-free virus-like particles (VLPs) was a breakthrough. It not only allowed for the large-scale production of HBV vaccines unable to infect host cells, but also created a blueprint for vaccines against other pathogens such as human papilloma virus (MILESTONE 14) and malaria and showed that a vaccine could be produced without the disease-causing pathogen itself. VLPs have also proved useful for applications such as antibody discovery, bioimaging and cell targeting. Recombinant DNA technology had lived up to its potential to transform basic research into applied research, whereby a living cell could be reduced to an information-processing machine and genetic engineering could become an integral part of both angles of research.

Mirella Bucci,
Nature Chemical Biology

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The sweet success of conjugate vaccines

Polysaccharide-encapsulated bacteria (such as *Haemophilus influenzae*, *Streptococcus pneumoniae* and *Neisseria meningitidis*) can cause serious bacterial infections, including bacterial meningitis and pneumonia, and have been a deadly scourge on humans for centuries. Before the introduction of effective vaccines in the 1980s, *H. influenzae* type b (Hib) was the leading cause of invasive bacterial disease in young children worldwide, affecting approximately 1 in 200 children under the age of 5 years in the USA. Even with the availability of antibiotic treatment, Hib infection resulted in thousands of deaths annually, necessitating effective prevention methods.

In the late 1960s, two groups, one led by John Robbins and Rachel Schneerson and the other led by Porter Warren Anderson and David Hamilton Smith, began independent investigations into the biology of Hib and potential vaccine strategies, a line of research that would eventually jointly earn these four researchers the prestigious Albert Lasker Clinical Medical Research Award in 1996.

Both research groups undertook the unusual strategy of focusing on the polysaccharide (sugar) capsule covering the surface of Hib, a structure that provides protection against host immune responses and is a major virulence factor. Given that the development of antibodies to this capsule was known to be crucial for acquiring immunity to Hib, they postulated that this polysaccharide capsule, in particular its primary component polyribosyl ribitol phosphate (PRP), could be leveraged as a vaccine. Such an approach differed notably from other vaccine strategies at the time, which mostly focused on using whole bacteria.

Several pure polysaccharide PRP vaccines were developed that provided some protection in adults and were subsequently licensed in the USA in 1985. However, these pure polysaccharide vaccines were ineffective in children under the age of 18 months, the age group most at risk of disease, and failed to induce immunological memory at any age owing to the T cell-independent nature of the PRP antigen response.

To overcome this issue, and drawing inspiration from work by Avery and Goebel in the 1920s, both groups independently developed a method for improving the immunogenicity of PRP by conjugating it to a protein carrier with strong antigenic properties, leading to the first protein–polysaccharide conjugate vaccines. Notably, such vaccines could induce features of T cell-dependent humoral immunity, including a memory response to booster doses of the vaccine.

The first invented and approved conjugate vaccine, developed by the group of Robbins and Schneerson, consisted of PRP conjugated to diphtheria toxoid (known as PRP-D). This vaccine was highly efficacious in Finnish infants and received FDA approval in 1987. Unfortunately, the vaccine was ineffective in Alaska Native infants, a population at high risk of disease.

Since the development of PRP-D, other more effective PRP conjugate vaccines that use different protein carriers (meningococcal outer membrane protein (PRP-OMP), CRM$_{197}$ (PRP-CRM) or tetanus toxoid (PRP-T)) have superseded PRP-D, leading to its withdrawal from the market in 2000. These PRP conjugate vaccines are now part of routine immunization schedules in many countries worldwide.

The introduction of PRP conjugate vaccines saw a rapid reduction in the number of cases of invasive Hib disease in multiple countries and in the past three decades has undoubtedly saved the lives of millions. The success of these vaccines inspired the development of other conjugate vaccines targeting various polysaccharide-encapsulated bacteria, including *S. pneumoniae* and *N. meningitidis*, and led to a renaissance in vaccine discovery that has rapidly changed the epidemiology of many childhood diseases and that continues to grow to this day.

Indeed, before the 2010s, *N. meningitidis* serogroup A accounted for the majority of cases of meningococcal disease in the meningitis belt of sub-Saharan Africa. The widespread introduction of a conjugate vaccine in the 2010s led to the virtual elimination of serogroup A in this high-risk region.

A more recent example is *Salmonella enterica*, a bacterium responsible for a serious and sometimes fatal complication known as typhoid fever. Two typhoid vaccines are currently available and recommended by the WHO: a live attenuated version of the bacterium (Ty21a) and a vaccine consisting of the purified capsular polysaccharide Vi (ViCPS). However, these vaccines are either unsuitable or are not immunogenic enough in young children, reminiscent of the experience with Hib in the 1970s.

A new typhoid conjugate vaccine, consisting of Vi conjugated to tetanus toxoid (Typbar TCV), has shown promising immunogenicity and safety results in clinical trials. This vaccine is currently licensed for private use in India and Nepal and received WHO prequalification in 2018. Multiple large studies of this vaccine in various Asian and African countries are currently ongoing. Only time will tell whether this vaccine mirrors the success of the first conjugate vaccines.

Jessica McHugh,
Nature Reviews Rheumatology

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 MILESTONE 13

Another layer of protection

Read any textbook on vaccination and you will learn that vaccines protect against their target diseases by inducing immune memory to specific pathogen components. However, interesting observations throughout vaccine history have suggested that some live vaccines offer additional benefits by protecting against unrelated infections. These early observations were dismissed or overlooked until a series of studies led by Peter Aaby in Guinea-Bissau, West Africa, in the late 1970s and 1980s showed that measles vaccination had beneficial effects on all-cause mortality that could not be explained by protection against measles alone. Since then, similar nonspecific effects have been reported for other types of live vaccine in both high-income and low-income regions of the world.

When the Bacillus Calmette–Guérin (BCG) vaccine against tuberculosis was introduced in the 1920s (MILESTONE 5), Albert Calmette noted that general mortality in vaccinated children was four times less than in unvaccinated children. Calmette concluded by asking whether BCG vaccination "confer[s] on the organism a special aptitude to resist those other infections which are so frequent in young children?" Carl Näslund noted similar effects on all-cause mortality after introduction of the BCG vaccine in Sweden in 1927. Writing in French, he was the first to

refer to nonspecific immunity ("une immunité non spécifique"), although he concluded that the effects were likely owing to selection bias.

As well as the BCG vaccine, Mikhail Chumakov and his wife Marina Voroshilova showed in clinical studies carried out in the Soviet Union in the 1970s that prophylaxis with oral polio vaccine (OPV) could reduce morbidity from influenza and other respiratory infections by 70–80%. But like Calmette and Näslund before, the nonspecific effects noted by Chumakov and Voroshilova were consigned to vaccine history.

Then in 1978, Aaby arrived in Guinea-Bissau. He observed that the very high measles fatality rate locally was independent of nutritional status, which contradicted the prevailing view that measles vaccination would have limited effectiveness because many of the children were too frail to survive in any case. The first measles vaccination campaigns ran in Guinea-Bissau in 1979 and 1980 and the results of these campaigns led Aaby to champion the concept of nonspecific effects of vaccines. By comparing the general mortality rate before and after vaccination in results published in 1984, he estimated a reduction of more than 50%. As measles normally caused 10–15% of all deaths in Guinea-Bissau, protection from measles alone could not account for this large reduction

> " **measles vaccine may confer a beneficial effect which is unrelated to the specific protection against measles disease** "

in mortality. Furthermore, there was little difference in vaccine efficacy against death when including or excluding death from measles. In an analysis published in 1995 of 10 cohort and two case–control studies from Bangladesh, Benin, Burundi, Guinea-Bissau, Haiti, Senegal and Zaire, Aaby concluded that "measles vaccine may confer a beneficial effect which is unrelated to the specific protection against measles disease."

Early detractors noted the observational nature of many of these studies, but the results have since been repeated for various live vaccines in a range of settings. For example, a population-based cohort study of Danish children involving Aaby's long-term collaborator Christine Stabell Benn showed that the live measles–mumps–rubella (MMR) vaccine was associated with reduced risk of hospital admission for any infection. Randomized controlled trials (RCTs) are difficult to carry out for vaccines that are already part of the routine schedule, but the recommendation for delayed BCG vaccination in low-birthweight infants in Guinea-Bissau enabled Aaby and colleagues to show in three RCTs that BCG vaccination at birth reduced neonatal mortality by 38% in low-birthweight infants compared with later vaccination.
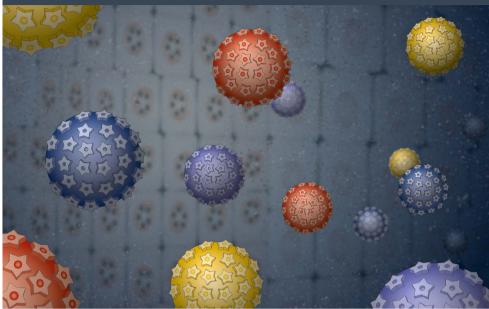
As a result of these studies, a systematic review sponsored by the World Health Organization concluded in 2016 that the BCG vaccine and measles vaccine have effects on mortality that are "more than would be expected through their effects on the diseases they prevent"— a reminder that we have much still to learn about the protective effects of vaccines.

Kirsty Minton,
Nature Reviews Immunology

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■━ MILESTONE 14

A vaccine to prevent HPV-related cancers

Credit: Carl Conway / Springer Nature Limited

In 1976, a German virologist, Harald zur Hausen, hypothesized that cervical cancers might be caused by a papillomavirus. Later work by his and other groups around the world demonstrated that human papillomaviruses (HPVs) were present in cervical cancer samples. Confirmation that infection with a 'high-risk' HPV is necessary for the development of cervical cancer raised the intriguing possibility that a cervical cancer-preventing vaccine might be developed.

A major roadblock to creating a cancer-preventing HPV vaccine, however, was that HPV could not be grown in the lab, and thus it was not possible to create a vaccine from attenuated or killed viruses, as was usually done. This changed in 1991, when a crucial technological advance was made by Ian Frazer and colleagues.

Frazer and colleagues used the then relatively new technology of expressing genes in cell culture to create virus-like particles (VLPs) of HPV16, a key cancer-causing high-risk HPV type. These VLPs formed spontaneously when the HPV16 capsid proteins L1 and L2 were expressed together (but not separately) from

a vaccinia virus expression vector in monkey kidney epithelial cells. Visualization of the VLPs by electron microscopy indicated that they had a virus-like 3D structure, unlike individually produced viral proteins, and it was hypothesized that VLPs would be more likely to induce an immune response in animals. Reporting their findings in the journal *Virology* in 1991, the authors recognized that VLPs "could provide a safe source of material for the development of a vaccine".

Eventually, these VLPs did just that. Efforts from groups led by John Schiller, Robert Rose and Toshiyuki Sasagawa, combined with ongoing work from the Frazer group, used more efficient gene expression systems in insect cells and yeast to produce larger quantities of HPV VLPs with the correct conformation. These VLPs were shown to induce antibodies in animals that were similar to those induced by infectious virus particles. Similar VLPs derived from non-human animal papillomaviruses were then used as the basis of experimental vaccines that induced antibodies that successfully prevented papillomavirus infection in animal models.

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> [VLPs] could provide a safe source of material for the development of a vaccine
> **"**

Clinical trials of HPV VLP vaccines of various HPV types indicated that they were safe and effective at preventing HPV infections. Then in 2006, the first HPV vaccine (Gardasil), containing VLPs of four HPV types — high-risk HPV16 and HPV18 (which cause ~70% of cervical cancers) as well as low-risk HPV6 and HPV11 (which cause genital warts) — was approved for use in the USA in adolescent girls. This was followed shortly after by approval of Gardasil or another VLP-based vaccine against HPV16 and HPV18 (Cervarix) in many other countries. Epidemiological studies in countries where vaccination is now routine and uptake is high have shown clear reductions in infections with the HPV types included in the vaccines, as well as in the development of cervical pre-cancerous lesions.

A newer version of Gardasil, which protects against five additional high-risk HPV types has now been approved. Furthermore, several other cancer types (including oral, head and neck, penile and anal cancers) have been attributed to HPV infections, so in many countries boys as well as girls now receive these vaccines. Although there are still issues in many countries with uptake of vaccination and access to these vaccines, the development of HPV VLPs and the recognition that they could be used to create vaccines has had a substantial public health impact that should only increase further as these vaccines become more widely adopted.

Sarah Seton-Rogers,
Nature Reviews Cancer

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Vaccinology in reverse

By the late 1990s, infections caused by serogroup B strains of *Neisseria meningitidis* (MenB), a major cause of meningococcal meningitis and septicaemia, had resisted all traditional vaccine development efforts. Vaccines for other meningococcal strains, based on their capsular polysaccharides, had been available since the 1960s; however, the capsular polysaccharide of MenB proved to be a poor immunogen owing to its similarity to a human autoantigen. Known antigenic proteins on MenB also proved unsuitable for vaccine development owing to their wide sequence variation.

Following the sequencing of the *Haemophilus influenzae* genome in 1995 by a team from the Institute for Genomic Research (TIGR), led by Craig Venter, researchers began to consider how sequencing could assist with the development of vaccines for pathogens such as MenB. A collaboration between a team at Chiron Vaccines, led by Rino Rappuoli, the TIGR and Oxford University produced two landmark papers published in *Science* in 2000, which annotated the whole genome of a MenB strain (MC58) and used this genomic information to identify a large number of novel surface-bound antigens. These reports, authored by Tettelin et al. and Pizza et al., represent the birth of 'reverse vaccinology' — the process of developing vaccines using a bottom-up approach from genome to vaccine.

Using the MC58 sequencing data published in Tettelin et al., Pizza et al. used a bioinformatics approach to identify open reading frames (ORFs) in the MC58 genome predicted to have features typical of genes encoding membrane-exposed proteins. A total of 350 candidate ORF sequences were amplified and cloned into expression vectors for His- or glutathione *S*-transferase (GST)-tagged proteins and expressed in *Escherichia coli*. Recombinant proteins were then purified and injected into mice, and immune sera from the mice were tested for the presence of specific antibodies through enzyme-linked immunosorbent

assay (ELISA) and fluorescence-activated cell sorting (FACS) analysis. Immune sera were also tested for bactericidal activity, with 28 proteins shown to produce bactericidal antibodies — a huge breakthrough considering only five antigens able to induce bactericidal activity had been identified in meningococcal species at that time.

Pizza et al. then analysed the seven ORFs associated with sera that were positive in all of the above assays, comparing these ORFs with genes from other *Neisseria* strains. Analysis of these ORFs showed they

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> These reports … represent the birth of 'reverse vaccinology' — the process of developing vaccines using a bottom-up approach
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Credit: sorbetto / Getty Images

were present in all 31 disease-associated MenB strains tested — and some in other pathogenic *Neisseria* spp. such as *Neisseria gonorrhoeae*. Whole-cell ELISA showed that antibodies specific to each of these antigens recognized all 31 of the MenB strains, suggesting that each protein was expressed and exposed at the bacterial surface and therefore accessible to host antibodies. These data therefore identified highly conserved, surface-exposed, immunogenic proteins on MC58. The authors noted that this approach allowed for the screening of antigens that may only have limited immunogenicity in a disease context and thus may be missed by traditional vaccinology approaches.

Following the success of this study, some of the antigens were selected for their capacity to induce broad protection; these antigens included *Neisseria* heparin binding antigen (NHBA), factor H binding protein (fHbp) and *Neisseria* adhesin A (NadA). A vaccine was developed consisting of these antigens and the highly variable membrane protein, PorA. After successful clinical studies, the vaccine (4CMenB; Bexsero®) was approved in Europe in 2013, before being introduced into the national immunization programme in the UK in 2015. It is now routinely used for immunization of newborns (less than 12 months of age) in the UK, and is also licensed for immunization of adolescents in the USA.

Since those studies in 2000, the speed of collecting genomic sequences has skyrocketed, and reverse vaccinology approaches have been applied to many other pathogens, including respiratory syncytial virus (RSV), human immunodeficiency virus (HIV), group A and group B *Streptococcus* species, and antibiotic-resistant strains of *Staphylococcus aureus* and *Streptococcus pneumoniae*. A limitation of the technique is that antigens cannot be graded in terms of their predictive ability for immunization; however, recent advances in B cell technologies and structural biology have allowed better characterization of the immunogenicity of antigens identified through reverse vaccinology. The capacity to generate human monoclonal antibodies from memory B cells and plasmablasts, for example, has allowed screening of antigens against human antibodies — improving the assessment and prioritization of bacterial epitopes as vaccine candidates. These advances have led to a new model for rational vaccine design: 'reverse vaccinology 2.0'.

Joseph Willson,
Nature Reviews Cross-Journal Team

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Credit: Simon Bradbrook / Springer Nature Limited

⊢⊞▸ MILESTONE 16

The quest for a vaccine against malaria

Malaria is mainly caused by *Plasmodium falciparum*, a eukaryotic parasite that is transmitted to humans through mosquito bites. A single parasite is able to initiate an infection. Worldwide, there are more than 200 million cases of malaria each year, with approximately 500,000 deaths. More than 80% of cases are in children under 5 years old and 90% of deaths occur in sub-Saharan Africa.

The high burden of malaria in Africa has persisted, despite continued preventive measures, owing to drug resistance in *P. falciparum* and the emergence of insecticide-resistant mosquitoes. A preventive vaccine has been a long-sought goal. The scientific proof of principle that malaria infection can be prevented following vaccination began with immunization studies of mice with attenuated (irradiated) sporozoites in the 1960s. In the 1980s, the identification of the circumsporozoite protein (CSP), the major protein expressed on the surface of the infecting sporozoite, which is essential for mediating liver infection, opened the door for development of a recombinant vaccine. CSP consists of an amino terminus, a central repeat region containing 39 NANP repeats and a carboxyl terminus that contains the T cell epitopes.

In 1987, scientists from GlaxoSmithKline (GSK) and the Walter Reed Army Institute of Research (WRAIR) used a truncated form of CSP linked to hepatitis B surface antigen (HBsAg) to produce RTS. RTS was then co-expressed in yeast cells with another free HBsAg to produce RTS,S. In 1997, an open-label trial showed that six out of seven healthy volunteers who received an RTS,S vaccine were protected against malaria. The study also reported that an adjuvant system, containing an oil-in-water emulsion with the immunostimulants monophosphoryl lipid A (MPL) and *Quillaja saponaria* fraction 21 (QS21), improved vaccine efficacy to 86% compared with 29% for adjuvant-free RTS,S. In 2001, a randomized trial in 306 adult men in The Gambia, Africa, showed 34% efficacy of RTS,S.

As children are particularly susceptible to malaria, in 2004, GSK and the Programme for Appropriate Technology in Health (PATH)-Malaria Vaccine Initiative conducted a double-blind, phase IIb, randomized controlled trial to examine the efficacy of RTS,S/AS02A (oil-in-water based adjuvant system containing MPL and QS21) in children 1–4 years of age in Mozambique, Africa. The vaccine efficacy was 29.9% for the first clinical episode and 57.7% for severe malaria. A similar randomized trial of 214 infants 10–18 weeks of age was carried out in Mozambique in 2007 for 6 months with a 3-month follow-up. It was shown that RTS,S/AS02D had a vaccine efficacy of 65.9%. These results indicated that development of an effective vaccine against malaria was feasible.

In 2011, a phase III randomized, controlled, double-blind trial of this vaccine with more than 15,000 children recruited from seven African countries showed that RTS,S/AS01 provided protection (~50% vaccine efficacy) against malaria in African children 5–17 months of age for up to 1 year. Of note, immune responses and protective efficacy were more limited in young infants 6–12 weeks of age. Extended follow-up revealed an efficacy of 28% against all malaria episodes over a median of 4 years, and 36% for those who had received a booster dose. These data show that while RTS,S/AS01 was relatively protective during the first months after administration, a gradual decline in efficacy was observed during extended follow-up. Further evidence for reduced protection over time came from a study from 2016 that investigated RTS,S/AS01 efficacy over a 7-year period, as part of a double-blind, randomized, controlled, phase II trial in 447 African children who were 5–17 months of age. The data showed that in the 5th year after vaccination, those vaccinated were less protected than those who had received a placebo.

A large-scale malaria vaccine implementation programme coordinated by the World Health Organization to investigate RTS,S/AS01 efficacy is now ongoing in Malawi, Ghana and Kenya. The programme aims to vaccinate about 360,000 children per year from 2019 to 2023, and will examine safety, compliance with the booster dose and reduction in mortality.

A key aspect in the development of the RTS,S vaccine was the recognition that adjuvants are important for enhancing protection. Furthermore, giving a delayed, fractionated dose of RTS,S/AS01 increased protection, and recent findings indicate that this involves improved T follicular helper cell and B cell responses. Durability of very high antibody titres remains a major obstacle for malaria vaccine efficacy, and future studies will need to assess how to keep antibody levels high through other adjuvants, vaccine delivery systems or vaccine regimens, or by including other epitopes to make additional neutralizing antibodies.

Recent potential alternatives to a vaccine include monoclonal antibodies, which could be used for inducing high-level, short-term protection that may apply to seasonal control or elimination campaigns. Nevertheless, the development of the RTS,S vaccine has substantially improved our chances of reaching the long-held goal of an efficient and safe vaccine against malaria.

Francois Mayer,
Nature Microbiology

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A dendritic cell cancer vaccine

Credit: Science Photo Library / Alamy Stock Photo

In 1909 Paul Ehrlich postulated that the immune system may defend the host against neoplastic cells and hinder the development of cancers. This concept has been widely recognized ever since, and eventually led to the development of novel cancer treatments in more recent years that revolutionized cancer care.

While the vast majority of cancer drugs target cancer cells directly, immunotherapies set off the body's own immune response against tumours. A complex network of cells and soluble factors can thus be mobilized as preventive and therapeutic cancer vaccines, monoclonal antibodies that reactivate an immune response, or immune cell-based therapies.

A common feature of cancer vaccines is the presentation of tumour-specific antigens (generated for instance by somatic mutations or oncogenic viruses) to immune cells to elicit an immune response against these cancer epitopes. Arguably the greatest success of cancer vaccines has been the development of vaccines against 'high-risk' strains of the human papillomavirus (HPV) for prevention of HPV-related cervical and other cancers (MILESTONE 14).

Dendritic cells, discovered in 1973 by the late Ralph Steinman, are the major antigen-presenting cells in the body, which, once activated, present antigens to CD4+ and CD8+ T cells and induce protective T cell responses. If a cancer-specific antigen is presented, this can result in an anti-tumour response. As T cell responses are indeed crucial for eliciting an immune response against cancers, dendritic cells have for a long time been suggested as potential cell-based vaccines. Crucial to the development of dendritic cells as vaccines, in the 1990s researchers developed the concept of loading, or 'pulsing', dendritic cells ex vivo with tumour-specific antigens.

The multi-centre phase III IMPACT trial reported in 2010, and two supporting phase III trials reported in 2006, showed a benefit to median survival, as well as induction of a T cell response, in patients with metastatic hormone-refractory prostate cancer who were treated with the dendritic cell-based vaccine sipuleucel-T (trade name Provenge), even though the time to disease progression was not altered. On this basis, in 2010, sipuleucel-T became the first approved dendritic cell cancer vaccine, for the treatment of late-stage prostate cancer.

Sipuleucel-T is a personalized treatment. Dendritic cell precursors are extracted from each patient and pulsed with a fusion protein of prostate acid phosphatase (PAP; an antigen present on most prostate cancer cells) and the cytokine GM-CSF, which helps antigen-presenting cells

> " Sipuleucel-T became in 2010 the first approved dendritic cell cancer vaccine "

to mature. The pulsed dendritic cells are then reinfused into the patient over several cycles.

Although sipuleucel-T has not been very widely adopted (and is no longer available in the European Union), it was recently announced that the combination of hormonal therapeutics with sipuleucel-T extended the survival of patients with metastatic castration-resistant prostate cancer. Other clinical trials combining sipuleucel-T with radiation, hormonal, targeted or other immunotherapies are ongoing. So far sipuleucel-T remains the only vaccine-based immunotherapy approved for prostate cancer, and is also the only approved cell-based vaccine in the USA.

Overall clinical responses to dendritic cell vaccines have been disappointing, but with increasing knowledge, newer and more sophisticated strategies are being investigated to improve the efficacy of dendritic cell-based vaccines. Improved methods to generate more mature and 'effective' dendritic cells using ex vivo protocols, alternative combinations of antigens, optimized loading of dendritic cells and transfection of dendritic cells with RNA or DNA are among the strategies under investigation. The exploration of dendritic cell subsets and of other agents beyond GM-CSF that may mobilize dendritic cells in vivo, such as FLT3L, are also being pursued.

One important consideration is that tumour-associated immunosuppression can hamper the efficacy of the vaccines. In more recent years, T cell therapies — and in particular antibody-based immunotherapies that disarm inhibitory immune cell interactions (so called immune checkpoint inhibitors) — have proved very successful for some patients across a wide range of cancer types. Vaccines designed to boost these treatments are now in combination trials and may yield even more effective immunotherapies.

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Nature

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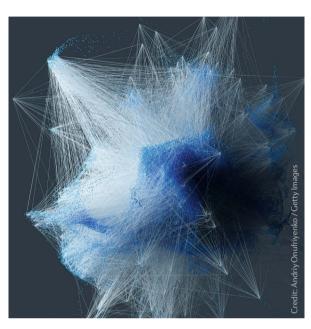
Assessing vaccine responses: you've got to have a system

As the number of available vaccines increased, so too did the desire to understand the effect that vaccination has on immune responses. Protective mechanisms such as the production of neutralizing antibodies and the induction of cytotoxic T cells were thought to be important, but the putative role of the innate immune system was uncertain and a holistic view that brought together all branches of the immune system was missing.

Systems biology, a field that has existed as a distinct entity since the 1960s, aims to describe the complex interactions between all parts of a biological system using large datasets and mathematical modelling, and can provide such a holistic view. By the late 2000s, advances in high-throughput biological techniques such as gene arrays and polychromatic flow cytometry, together with the development of computational analysis methods, put researchers in a position to offer a viable systems biology approach to the interrogation of immune responses to vaccination.

Two seminal papers were published online in *Nature Immunology* and the *Journal of Experimental Medicine* in 2008 that assessed how the immune system responds to the live attenuated yellow fever 17D (YF17D) vaccine. The potency of YF17D, which was first developed in the 1930s (MILESTONE 7), made it the perfect candidate with which to model innate and adaptive immune responses to vaccination.

In their *Nature Immunology* paper, Bali Pulendran and colleagues set out to identify innate immune signatures that could be used to predict subsequent adaptive immune responses using a combination of multi-parameter flow cytometry, multiplexed chemokine and cytokine analysis, gene expression analysis and computational modelling. This multi-pronged approach enabled them to identify a gene signature that could

Credit: Andriy Onufriyenko / Getty Images

predict an individual's CD8^{+} T cell response with 90% accuracy and another distinct signature that could predict their neutralizing antibody response to the vaccine with 100% accuracy. These results were the first indication that computational modelling approaches (and machine learning in particular) could be used to predict an immune response to vaccination.

Pulendran and colleagues also revealed important roles for components of the innate immune system, such as complement, Toll-like receptor 7 (TLR7) and the type I interferon signalling pathway, in the response to YF17D. These findings were echoed in the publication from Rafick-Pierre Sékaly's group in the *Journal of Experimental Medicine*.

Sékaly and colleagues used a combination of functional genomics and polychromatic flow cytometry to study immune responses up to 1 year after vaccination with the aim of defining the signature of the immune response to YF17D. Their results highlighted the importance of the innate immune system, corroborating data from Pulendran and

> ❝ systems biology approaches might now have become routine as a way of monitoring immune responses in vaccine clinical trials ❞

colleagues on complement, TLR7 and type I interferons, and adding to that a potential role for inflammasomes. Sékaly and colleagues also reported evidence of an early, mixed effector T cell response that was followed by a somewhat variable B cell response. However, unlike Pulendran and colleagues, Sékaly and colleagues did not use machine learning to predict an individual's immune response to vaccination in an independent trial.

These two papers heralded the beginning of systems vaccinology as a field of research. Subsequent studies using similar systems biology approaches have been used to predict immune responses to other vaccines, including the seasonal influenza vaccine. The large datasets required for these studies have encouraged large-scale collaborations and ambitious projects to model the human immune system. One such study constructed computational models to predict antibody responses to influenza vaccination purely on the basis of pre-vaccination immune system parameters — a feat unthinkable 20 years ago.

The use of systems biology approaches might now have become routine as a way of monitoring immune responses in vaccine clinical trials, but these approaches are still being used to produce hypothesis-generating data that have considerable implications for vaccinology and immunology. For example, a 2019 systems biology paper was the first to demonstrate the importance of the gut microbiota in the generation of immune responses to vaccines in humans, which could have an effect on the way that vaccines are delivered to individuals taking antibiotics. This ability to provide data of relevance to both basic and clinical research sets systems vaccinology apart and holds hope for future discoveries that will continue to improve vaccine development and testing.

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Public trust in vaccines

Satirical artwork from 1802 by James Gillray, showing the supposed effects of using cowpox as a vaccine against smallpox.
Credit: GL Archive / Alamy Stock Photo

In early February 2010, *The Lancet* medical journal retracted a case study it had published 12 years earlier. The retracted study was led by the English physician Andrew Wakefield and claimed to have identified a new 'autistic enterocolitis' syndrome in 12 children. Without providing any supporting data, in the discussion section of the article, the authors proposed a causal link between immunization of these children with the measles, mumps and rubella (MMR) vaccine and the development of this syndrome. Numerous studies have since discredited the idea that the MMR vaccine causes autism, with no evidence of this found in multiple large-scale studies, including one in Denmark that involved more than half a million children. Moreover, subsequent investigations identified major faults in the conduct of the original Wakefield study, and he was later struck off the UK medical register.

Still, by this point the damage had been done. Widespread media coverage of the Wakefield study drove fear and anxiety in parents, causing vaccination rates to plummet. This has contributed to measles outbreaks throughout the world in countries that had previously achieved herd immunity to this dangerous virus. Scepticism of the MMR vaccine persists to this day — in 2019, the UK lost its 'measles-free' status with the WHO.

Unsubstantiated health scares have affected other vaccines too. In England and Wales, rates of childhood immunization with the diphtheria–tetanus–whole-cell-pertussis (DTwP)

> ❝ the refusal of perfectly safe and effective vaccines is a worrying trend ❞

vaccine fell from 78.5% to 37% in the mid-1970s after the whole-cell-pertussis component was suggested to cause brain damage. In fact, although the cellular pertussis component was shown to cause minor adverse reactions in some children, it was never proved to cause serious neurological damage. However, the loss of public confidence led to a major whooping cough epidemic in the late 1970s and the eventual replacement of DTwP with newer vaccines containing an acellular pertussis component.

An effective vaccine against Lyme disease was licensed by the FDA in 1998 but withdrawn from the market in 2002 after it was wrongly claimed to cause autoimmune side-effects. Anti-vaccine propaganda has affected uptake of the human papillomavirus (HPV) vaccine, which protects against cervical cancer. HPV vaccination rates in Japan plummeted from more than 70% in 2010 to less than 1% in 2013 after the government suspended their proactive recommendation of the vaccine owing to public safety concerns. Although the reported adverse reactions were later investigated and found not to be caused by the vaccine, the government suspension has not been repealed and around 25,000 cases of cervical cancer and more than 5,000 deaths

have been attributed to the drop in vaccination. Geopolitical tensions can also contribute to vaccine hesitancy. The false belief that polio vaccines were contaminated with oestradiol as part of a US-led plot to cause infertility in Muslims prompted the Kano state government in Nigeria to suspend polio vaccination between 2003 and 2004. This caused a resurgence of polio in Nigeria and neighbouring regions, even as far as Indonesia.

Negative public perception of vaccination is not a modern-day phenomenon. In 1802, the English satirist James Gillray depicted the unfortunate recipients of Edward Jenner's cowpox vaccine with bovine projections emitting from their skin and various orifices. Jenner and other early advocates of inoculation also faced theological opposition. A sermon by the Rev. Edmund Massey in 1772 (some 24 years before Jenner's vaccination of James Phipps) denounced the 'dangerous and sinful practice of inoculation'. Massey preached that 'diseases are sent... for the punishment of our sins'. Even today, parents can refuse otherwise mandatory vaccines on the grounds of religious beliefs.

In the midst of a global pandemic, the issue of public confidence in vaccination is more urgent than ever. The WHO has described vaccine hesitancy as one of the top ten threats to global health. Assuming that scientists develop an effective vaccine against COVID-19, can we be sure the public will want to use it? A recent study in *Nature* that analysed Facebook interactions found that anti-vaccine clusters are more effective than pro-vaccine clusters in engaging with undecided groups. Rather ominously, the study predicted that anti-vaccination views could dominate within a decade. It seems that despite hundreds of properly designed studies supporting the safety and efficacy of vaccines, unfounded opinions on social media can have more traction.

It is important to acknowledge the valid safety concerns that surround some vaccines. However, the refusal of perfectly safe and effective vaccines is a worrying trend. It calls for scientists, politicians and educators to work together to build and maintain public trust.

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MILESTONE 20

Synthetic biology speeds vaccine development

The 2009 influenza pandemic prompted the fastest global vaccine development effort in history. But it wasn't fast enough.

By the time vaccine companies had designed, tested and distributed hundreds of millions of doses of licensed vaccines — a process that, using the best technologies available, took about 6 months — the pandemic wave had already swept across the world.

Wanting to speed up the development clock, scientists at Novartis teamed up with collaborators at the J. Craig Venter Institute and Synthetic Genomics and, using synthetic biology techniques, devised a way of turning genetic sequence data from a novel virus into a vaccine candidate in a matter of days.

Instead of using killed or weakened viruses, as most vaccine developers had done in the past, the Novartis-led team planned to deliver carefully designed RNA segments, which would instruct cells in the body to create a protein that imitates part of the target virus and primes the immune system to attack if the real virus enters the cell.

> **The speedy development… set the stage for current efforts to rapidly address the outbreak of novel coronavirus disease**

In 2011, the researchers beta-tested this novel vaccine platform in response to a mock influenza pandemic. That preparation then paid off richly when, 2 years later, in March 2013, Chinese health officials announced three cases of people infected with a novel strain of avian influenza.

The same Novartis-led team jumped into action.

The researchers downloaded the virus's gene sequences from the internet. Within a week, they had chemically synthesized the genes encoding the vaccine antigens and created a fully synthetic RNA-based vaccine that was ready for preclinical testing. They also inserted the same gene sequences into a genomic backbone common to many flu viruses to fashion an inactivated-virus vaccine that entered human testing in August 2013.

That vaccine candidate quickly proved safe and immunogenic. And by the end of the year, Novartis had already begun mass-manufacturing the vaccine, allowing the US government to amass a strategic reserve of the product.

Fortunately, that strain of influenza did not become a global pandemic. But the speedy development, clinical testing and stockpiling of an effective synthetic vaccine against bird flu set the stage for current efforts to rapidly address the outbreak of novel coronavirus disease.

The possibility of using synthetic genes for rapid vaccine development against emerging infections also became one of the main goals of the Coalition for Epidemic Preparedness Innovations, a global partnership established in 2016. Plus, synthetic genes paved the way for oncology-focused companies to generate cancer vaccines individualized to the specific DNA sequence of a patient's tumour.

Several such personalized cancer vaccines are now in clinical testing. More than a dozen gene-based vaccines are also in the works to fight COVID-19.

Notably, the scientists behind all those experimental coronavirus vaccines have essentially followed the same playbook established by the Novartis-led researchers years earlier: they each started with genomic data from the mysterious new virus first reported in Wuhan, China, and — informed by 3D protein structures deciphered soon thereafter — worked at record-breaking speeds to make candidates available and ready for clinical testing.
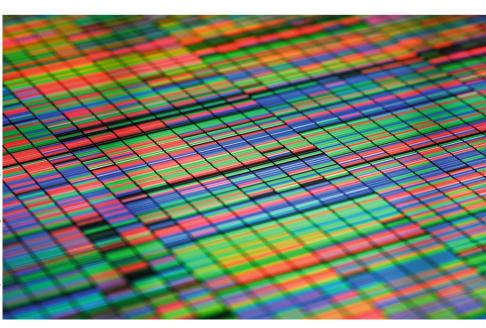
On 16 March 2020, just 66 days after the viral genome was released, clinicians administered the first dose of the first vaccine candidate in a first-in-human trial. Others soon followed.

As the race for a COVID-19 vaccine intensifies, time is clearly on the side of synthetic biology. But will the approach ultimately prove safe and effective? Only time will tell.
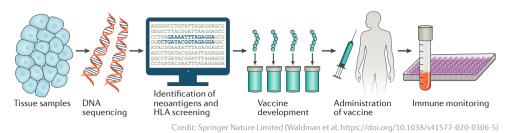
Elie Dolgin

Credit: Kiyoshi Takahase Segundo / Alamy Stock Photo

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Tissue samples — DNA sequencing — Identification of neoantigens and HLA screening — Vaccine development — Administration of vaccine — Immune monitoring

Credit: Springer Nature Limited (Waldman et al. https://doi.org/10.1038/s41577-020-0306-5)

MILESTONE 21

Individualized neoantigen vaccines

The immune system is recognized mostly by its role in protecting from infectious pathogens, but a perhaps less obvious function of immune cells is in surveying the body to find and eliminate transformed cells (i.e. cancer). Because of the inbuilt capacity of the adaptive immune system to recognize foreign proteins, adaptive immune cells can recognize mutated tumours displaying so-called neo-antigens, which are former self-proteins with changes in their peptide sequence no longer recognized as endogenous. So, if one can artificially trigger immune responses to pathogens through immunizations, why not vaccinate against tumours?

Cancer vaccines have indeed been developed, and the strategies employed are varied and mimic the approaches used for developing vaccines against infectious pathogens. From formulations based on tumour cell extracts, to strategies based on dendritic cells loaded with tumour antigens (MILESTONE 17), to administration of the purified mutated tumour antigens themselves, featuring multiple delivery systems and adjuvants, preclinical research of a wide range of formulations has been met with varying levels of success in animal models.

But a significant limitation of developing a cancer vaccine versus developing a vaccine to a bacterium, for example, is that while bacteria are totally foreign entities, completely made of non-human proteins, tumour cells retain most of the endogenous proteins and are thus mostly tolerated by the immune system. The challenge is then to identify neoantigens — originally self-proteins that, through the acquisition of mutations, generate new molecular epitopes recognized as foreign by the immune system — for each patient.

Following several reports in mouse cancer models of mounting anti-neoantigen immune responses through vaccination, a small phase I trial in 2015 described enhancement of neoantigen-specific immunity in three patients with advanced melanoma who were immunized with dendritic cells loaded with a mixture of melanoma neoantigens. Although the trial was not designed to assess patient outcomes, it showed a way to effectively boost the immune system towards tumour-specific antigens. It is worth noting that melanoma is especially amenable to a neoantigen vaccine approach owing to its heavy mutation burden, which facilitates neoantigen identification and makes the tumour inherently more susceptible to an antigen-specific immune response.

About 2 years after this landmark paper, two reports published in *Nature* took the strategy further, describing the vaccination of patients with advanced malignant melanoma with neo-epitopes. In one of the studies, Catherine Wu and colleagues devised a vaccine consisting of peptides 13–20 amino acids long containing predicted personal tumour neoantigens for administration to patients who had prior surgical tumour resection; in four of the six patients immunized, no disease recurrence was observed at 25 months after vaccination. In the other study, Ugur Sahin and colleagues followed a different vaccine formulation, in that they used an RNA-based poly-neo-epitope suspension instead of synthesized peptides; also in this study, vaccinated patients developed T cell responses against multiple vaccine neo-epitopes with a reduction in the rate of metastatic events.

These first studies are important because they show a possible approach

> A main challenge in taking cancer vaccines mainstream will be optimizing the complex manufacturing pipeline

for boosting antitumour immunity that is safe and potentially effective. Perhaps more importantly, it can be expected that cancer vaccines complement other immunotherapy modalities well — particularly immune checkpoint blockade, as the two approaches follow orthogonal immune mechanisms. Indeed, the two studies suggest a benefit from combining either vaccine formulation with immune checkpoint inhibition.

A main challenge in taking cancer vaccines mainstream will be optimizing the complex manufacturing pipeline that enables personalization. Neo-epitope prediction and identification are based on next-generation sequencing data that require processing by a range of bioinformatics tools, such as those for the prediction of neo-epitope binding to human leukocyte antigen molecules that determine antigen presentation. Current manufacturing protocols that enable individualized vaccine production under good manufacturing practices still take several months, and are costly.

Other difficulties are biological in nature: many tumour types (such as neuroblastoma, pancreatic cancer and prostate cancer) have a low mutational burden, which hinders the identification of neoantigens. To optimize doses and combinations with alternative therapy modalities to maximize efficiency, patient and tumour heterogeneity will need to be taken into account. In this regard, patient stratification and integration of response predictors may be necessary.

In the context of all the efforts to create off-the-shelf therapies, the challenge of designing a vaccine for each individual patient may seem herculean. But because it is based on the exquisite specificity inherent to the adaptive immune system, cancer vaccines offer a level of targeting that is still out of reach of most other cancer therapies in the clinic today.

João H. Duarte,
Nature Biomedical Engineering

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