TIMELINE

The development of vaccines: how the past led to the future

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Abstract | The history of vaccine development has seen many accomplishments, but there are still many diseases that are difficult to target, and new technologies are being brought to bear on them. Past successes have been largely due to elicitation of protective antibodies based on predictions made from the study of animal models, natural infections and seroepidemiology. Those predictions have often been correct, as indicated by the decline of many infections for which vaccines have been made over the past 200 years.

It is said that only those who have seen the beginning of things can understand the present. As the development of vaccines continues in the twenty-first century, and as it is now over 215 years since vaccinology was launched by Edward Jenner's observations of the powers of cowpox to prevent smallpox, it is useful to contemplate the past. This is all the more true because there is a great deal of forward gazing, with an explosion of new potential strategies for vaccine development based on genetic engineering, and the hope that systems biology and structural biology will tell us which genes must be upregulated or downregulated and what antigenic constructions are needed to achieve a protective immune response[1–4]. However, as the future unfolds, the past is sometimes deprecated, a fact that is conveyed in recent expressions by two distinguished individuals: "What happened in the past is that most vaccines have been made empirically without a real immunologic rationale" (REF. 5) and "We really don't know how to make vaccines in a predictable way. It's still a little bit of black magic" (REF. 5). Although those statements are true of the early history of vaccines, they have not been true for most of the twentieth century, as we show below.

The beginning

First, let us return to the 1700s, when both the farmer Benjamin Jesty and the physician Edward Jenner paid attention to the unsullied complexions of milkmaids and inferred that cowpox protected them from the ravages of smallpox. Jesty inoculated his own family[6], but Jenner carried out what passed for clinical trials in the eighteenth century and then broadcast the results to the world[7,8]. There ensued a rapid spread of inoculation, using material obtained from poxvirus lesions on the arms of humans[9,10]. To this day, we do not know the origin of the virus that Jenner called vaccinia, which may have been a now-extinct strain of horsepox[11,12], but its use was adopted in all parts of the world, culminating in the eradication of smallpox.

However, 80 years were to pass before the next step in the history of vaccines, which was taken in the laboratory of Louis Pasteur. The story that his discovery of attenuation — using the causative organism of chicken cholera, now known as *Pasteurella multocida* — was an accident has gained currency[13,14]. That story is disputed but, whether by accident or premeditation, Pasteur learned that he could attenuate a bacterium by exposure to adverse conditions. His work on anthrax and rabies followed from that discovery[15,16], but his theoretical basis for attenuation was completely wrong. Pasteur thought that resistance was due to the depletion of an element that was crucial to the growth of an organism. Nevertheless, although he did not understand what the vaccines were doing, the practical results achieved were epochal.

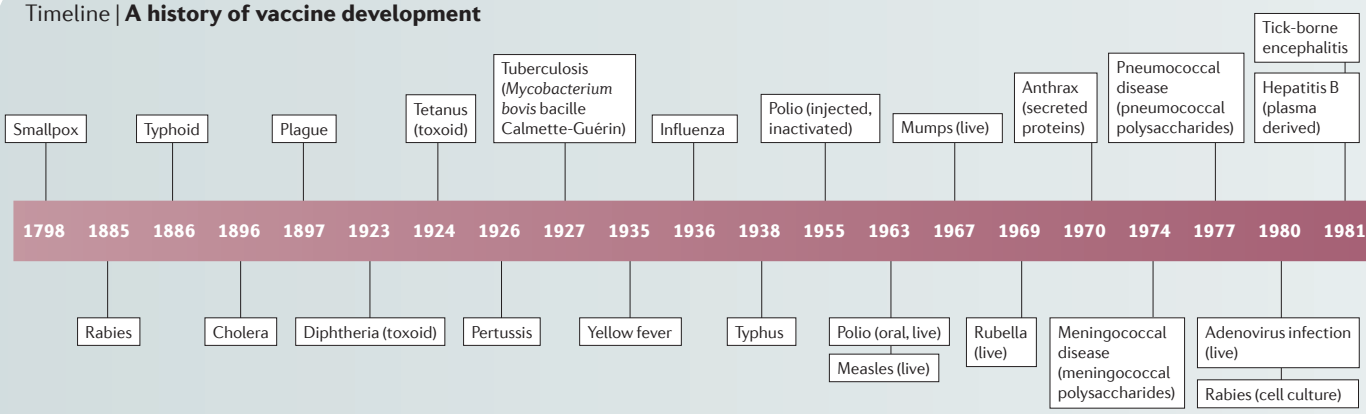
In the last decade of the nineteenth century, vaccine development started to have a rationale. The science was produced by workers in Great Britain, Germany, the United States and Pasteur's laboratory in France. The key developments were methods to inactivate whole bacteria, which could then be used as vaccines, the discovery of bacterial toxins, the production of antitoxins and the realization that immune serum contained substances (antibodies) that neutralized toxins or bacterial replication. Daniel Salmon and Theobald Smith were the first to inactivate bacteria, and wrote: "Immunity is the result of exposure of … the animal body to the chemical products of the growth of specific microbes which constitute the virus of contagious fever." (REF. 17.) Furthermore, during the last years of the nineteenth century and the beginning of the twentieth, inactivated whole-cell vaccines against typhoid[18], cholera[19] and plague[20] were produced and tested.

The key workers responsible for developing the concept of serum antibody include Emil von Behring, Shibasaburo Kitasato, Émile Roux, Alexandre Yersin, Almwroth Wright and Paul Ehrlich. In 1888, Roux and Yersin demonstrated that diphtheria bacilli produce an exotoxin[21], and 2 years later von Behring and Kitasato showed that an antitoxin was induced in the sera of animals that had received sublethal doses of the toxin[22]. Von Behring summarized both the practical and theoretical facets of the work by saying: "Briefly expressed, serum therapy works through antibodies." (REF. 23.) Ehrlich, in particular, thoroughly developed the concept of antibody as being complementary to antigen[24].

In 1923, Alexander Glenny and Barbara Hopkins showed that diphtheria toxin can be converted into a toxoid by the action of formalin[25]. Its toxicity was thus reduced, but it was well tolerated only in combination with antitoxin. A stable, non-toxic, formalin-inactivated diphtheria antigen was finally produced by Gaston Ramon[26].

In the early years of the twentieth century, it became clear that the passage of organisms in unnatural hosts results in genetic selection for avirulent strains. Thus, the *Mycobacterium bovis* bacille

Timeline | **A history of vaccine development**



*Capsular polysaccharide conjugated to carrier proteins. ‡Killed, recombinant B subunit, whole-cell vaccine. §Cholera toxin B combined with enterotoxigenic *Escherichia coli*. ||Now withdrawn.

Calmette–Guérin vaccine was obtained by 230 serial passages of *M. bovis* over a period of 14 years, on artificial medium containing bile. Albert Calmette and Camille Guérin demonstrated that the resulting mutant protected animals and infants against *Mycobacterium tuberculosis*, although the basis for protection was unknown[27,28].

Viruses

Filterable agents, which were subsequently called viruses, were also described in the last years of the nineteenth century. At this time, yellow fever was an important problem in Africa, and many scientists sought to attenuate the virus. The yellow fever virus strain 17D was selected from a virulent strain by Max Theiler by serial passage in minced chicken embryo and then in embryonated chicken eggs[29,30]. The goal was to eliminate neurovirulence, and for animals this was lost between the eighty-ninth and one hundred and seventy-sixth passages, but the attenuated virus still elicited neutralizing antibodies that protected monkeys from challenge with a virulent virus[29–31]. The vaccine made with yellow fever virus strain 17D became a major public health success.

At about the same time, two additional vaccines came into use: the whole-cell *Bordetella pertussis* vaccine and the influenza virus vaccine. The first *B. pertussis* vaccines were composed of inactivated whole bacterial cells, which induced agglutinating antibodies[32,33]. Later in the twentieth century, the bacterial antigens that induced protective antibodies were identified, and acellular vaccines containing 1–5 of these proteins replaced the whole-cell *B. pertussis* vaccine in many countries[34,35].

Wilson Smith, one of the discoverers of influenza virus, used the ferret as an experimental animal to show that prior infection by influenza virus induces immunity to future challenge[36]. However, by the 1940s it was clear that there is more than one influenza virus strain and that antigenic variation occurs frequently, rendering earlier vaccines ineffective[37]. Routine vaccination with inactivated influenza virus or, later, with viral haemagglutinin is based on the protection afforded by haemagglutination-inhibiting antibodies. Nevertheless, antigenic variation continues to be a problem, and current research is directed towards finding conserved antigens. In addition, building on the work that was initially carried out in the former Soviet Union[38], an intranasally administered live attenuated influenza virus vaccine is now in use[39]. This vaccine is effective because it induces secretory immunoglobulin A in the nasopharynx and serum, as well as cytotoxic T cell responses against the virus.

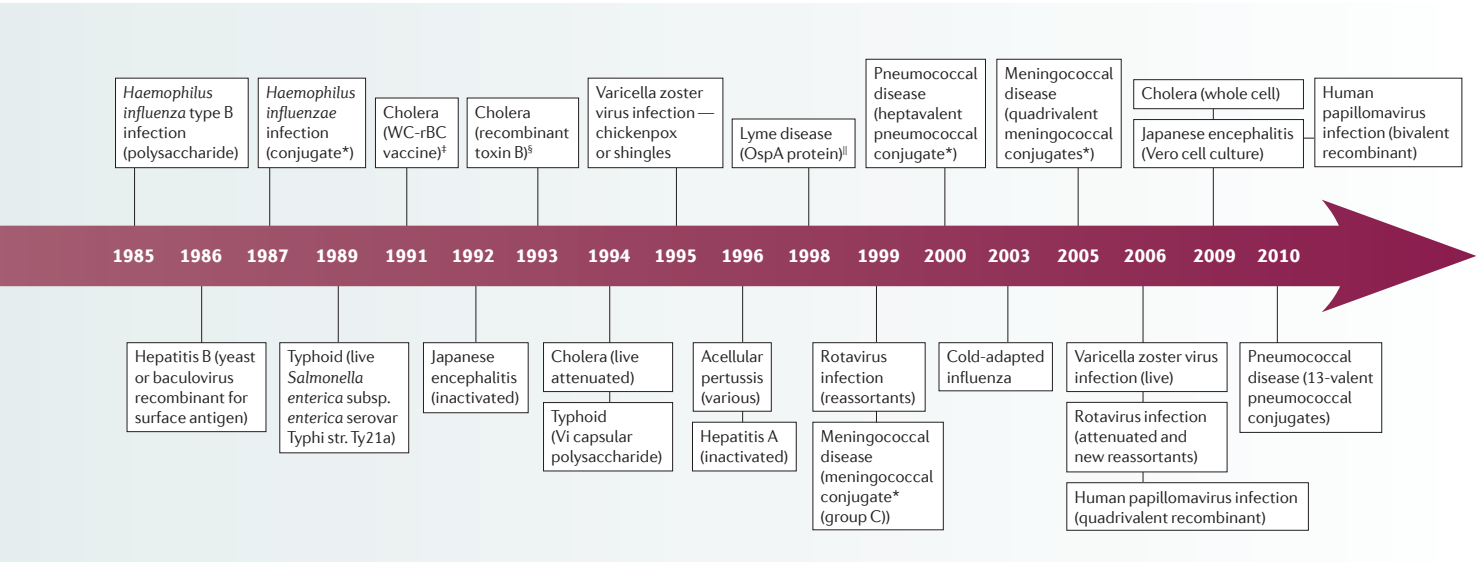
> “In the last decade of the nineteenth century, vaccine development started to have a rationale.”

The golden age

The golden age of vaccine development was launched by a methodological breakthrough in the mid twentieth century: the growth of viruses in cell culture. The pioneers of this technique were John Enders, Frederick Robbins and Thomas Weller, and their method was rapidly applied to vaccine development[40]. The 1950s was the era of the great poliovirus vaccine controversies, during which both an inactivated vaccine and a live vaccine were developed, the former by Jonas Salk[41] and the latter by Albert Sabin[42]. Salk had learned about virus inactivation from his prior work on the influenza virus vaccine, and Sabin's selection of attenuated mutants in cell culture followed Hilary Koprowski's attenuation of poliovirus type 2 by passage in mice[43]. In both cases it was understood that antibodies against the three types of poliovirus would protect individuals, because prior successful trials had demonstrated the prophylactic power of immunoglobulins[44].

In the 1960s, three classical attenuated-virus vaccines were developed: against measles virus, by Samuel Katz and John Enders[45]; mumps virus, by Maurice Hilleman[46]; and rubella virus, by several workers (including S.A.P.)[47–49]. These were all developed by passage in embryonated eggs or cell culture, and in the case of rubella virus, passage in cells incubated at 30°C selected for attenuation. In all three cases, it had been established using passive administration that the presence of neutralizing antibodies correlated with protection, so the aim was to render the viruses less reactogenic but maintain their immunogenicity.

In the 1970s, those principles were applied to the varicella zoster virus vaccine by Michiaki Takahashi, who attenuated the virus by passage in guinea pig cells[50]. As the vaccine induces both antibodies and cellular immune responses against the virus, similarly to natural infection, the efficacy of the vaccine was predictable. In addition, an



inactivated Japanese encephalitis virus vaccine based on production in mouse brain[51], a tick-borne encephalitis virus vaccine produced in cell culture[52], and a live, auxotrophic strain of typhoid bacillus[53] were licensed. In all three cases, serum antibodies against the organism were elicited and correlated with protection.

The 1980s saw the birth of two important strategies for vaccine development: the conjugation of bacterial capsular polysaccharides to proteins, and genetic engineering. In fact, the use of protein conjugation to improve the immunogenicity of polysaccharides had been devised years before — by Oswald Avery and Walther Goebel in 1931 (REF. 54) — but it was not put to good use until much later. Capsular polysaccharides from *Haemophilus influenzae* type b[55], groups A, C, Y and W135 meningococci[56] and multiple serotypes of pneumococci[57,58] were turned into bacterial vaccines (by Porter Anderson and David Smith, Emil Gotschlich, and Robert Austrian, respectively) because they induced opsonophagocytic antibodies and because it had been shown that the presence of those antibodies coincided with natural immunity. However, it soon became apparent that the polysaccharides induced poor B cell memory and failed to induce functional antibodies in infants. Robbins and Rachel Schneerson, along with their associates, launched the conjugate-vaccine era by coupling diphtheria toxoid to the *H. influenzae* type b capsule[59]. Soon, conjugation with diphtheria or tetanus toxoids was also used to develop potent vaccines against meningococci[60] and pneumococci[61]. In countries that use these conjugate vaccines, the diseases caused by meningococci and pneumococci have been almost eliminated. A capsular polysaccharide vaccine against typhoid was also licensed[62,63], and attempts are being made to improve it by protein conjugation.

A highly effective vaccine against hepatitis A virus was developed by classical inactivation of the whole virus[64]. Its success was also predictable, owing to the high efficacy of antibodies against the virus. Similarly, several cell culture-derived rabies virus vaccines have been developed that contain inactivated virus and induce antibodies against the virus[65]. These antibodies neutralize the virus at the site of the bite and thus block its attachment to the axons of neurons.

> “The golden age of vaccine development was launched by a methodological breakthrough in the mid twentieth century: the growth of viruses in cell culture.”

Genetic engineering

Genetic engineering, first conceived by Stanley Cohen and Herbert Boyer (allegedly after a conversation in a delicatessen), has allowed bacteria, yeast, animal cells and insect cells to become substrates for the production of immunogenic proteins — for insect cells, through the use of baculovirus vectors[66]. The first vaccine to be developed through genetic engineering was against hepatitis B virus. Maurice Hilleman had previously made a vaccine by purifying and inactivating hepatitis B virus antigen particles from infected individuals, but this method carried an obvious safety risk[67]. Nevertheless, this plasma-derived vaccine established the fact that antibodies against the antigen protect against infection. The development of genetic engineering at the University of California, USA, and at Genentech allowed new hepatitis B virus vaccines to be made by expressing the gene for the surface antigen in one of the available cellular substrates[68].

Other important licensed vaccines that were developed through genetic engineering are those against human papillomaviruses. In this case, the genes encoding the L1 proteins of oncogenic serotypes were inserted into yeast or baculoviruses for the production of virus-like particles[69,70]. Evidence obtained with animal papillomaviruses showed that antibodies prevent viral attachment to the basement membrane and subsequent infection of basal cells, thus blocking the eventual transformation of cells to malignancy; on the basis of this finding, the vaccines against human papillomaviruses could be developed.

A vaccine against Lyme disease containing a *Borrelia burgdorferi* surface antigen (OspA) that was produced in *Escherichia coli* was briefly on the market in the United States. Antibodies induced by the antigen inactivate the bacterium in the tick, which is why that antigen was chosen for the vaccine[71,72]. Efficacy correlating with antibody titres was demonstrated in field trials.

Two other important vaccines to be licensed recently are directed against rotaviruses. These vaccines are live viruses — in one case, a pentavalent combination of serotypes[73], and in the other, a single strain[74]. However, the success of both vaccines was based on two important facts: prior natural infection results in immunity to disease, and two viral proteins on the surface of the virus

Box 1 | **Adjuvants**

The word adjuvant is derived from the Latin *adjuvare*, meaning 'to help'. Adjuvants are supposed to increase the adaptive immune response to antigens. Until recently, the only adjuvants used in licensed vaccines were aluminium salts. It was thought that they caused a depot effect at the site of the injection, such that the adjuvant allows the slow release of antigens over time, but now it has been realized that they act as inflammatory agents. The development of new adjuvants is a burgeoning field, in part owing to the discovery of cellular receptors that react to danger signals. The first of these to be characterized were the Toll-like receptors (TLRs), and adjuvants that stimulate TLR4 have been licensed. In addition, several oil-in-water adjuvants have been incorporated into vaccines to increase antibody responses.

Many other TLR agonists are being developed for vaccine enhancement, such as CpG oligonucleotides, flagellin and double-stranded RNA. In addition, it has been recognized that cellular cytokines can be used to enhance immune responses or to direct them towards T helper 1 or T helper 2 pathways; interleukin-12 and granulocyte macrophage colony-stimulating factor (GM-CSF) have featured notably in this regard.

As vaccinology moves towards the development of purified protein and peptide antigens, the use of strong adjuvants becomes more and more important in order to stimulate innate immune factors that in turn augment B cell and T cell expansion, leading to enhanced adaptive immunity.

induce neutralizing antibodies. Although the important antibody isotype is almost certainly IgA, and it may be that responses at the intestinal level to other antigens are important for protection, both vaccines induce IgG and IgA antibodies as a result of oral administration and are highly effective.

The role of immunology research

This brief history shows that since the middle of the twentieth century, or even before, successful vaccine development has been based on an understanding of which immunological response is protective, and this has usually been serum and/or mucosal antibodies. However, two vaccines depend on cellular immunity, a concept that was first described by Élie Metchnikoff[75]. This concept became clearer with the separation of lymphocytes into B cells and T cells by Jacques Miller[76] and Robert Good[77]. Now, the importance of T cells after vaccination with the *M. bovis* bacille Calmette–Guérin and varicella zoster virus vaccines is well recognized. The zoster vaccine, which is composed of large amounts of attenuated virus, protects the recipient because the cellular immune response against the virus reduces with age and the vaccine reawakens this response[78]. In addition, T helper cells are essential for the development of B cell memory and long-lasting antibodies following vaccination with other vaccines.

Thus, it has been understood for some time that live agents can be attenuated by passage under unfavourable conditions to induce the selection of mutants, and that inactivated vaccines can be constructed with carbohydrates and proteins that are either separated from the microorganism or on the surface of killed pathogens. It has also been understood that, for both types of vaccines, the goal is the induction of antibody and T cell responses.

Box 2 | **New strategies for vaccine development**

Attenuated vaccines

- Reverse genetics, temperature-sensitive mutations and reassortment.
- Viral recombinants and deletion mutants.
- Codon de-optimization.
- Control of replication fidelity.
- MicroRNA insertion.
- Replicating vectors that contain genes from pathogens.
- Gene delivery by invasive bacteria.

Inactivated vaccines

- DNA plasmids and DNA shuffling.
- Reverse vaccinology.
- Antigen identification by transcriptomics and proteomics.
- Development of fusion proteins.
- Development of new adjuvants (including cytokines).
- Induction of innate immunity.

Certainly, new knowledge of innate immunity, of the different types of antibody functions, and of both $CD4^+$ and $CD8^+$ T cell functions in response to vaccination is critically important. Reverse vaccinology and structural biology will help us to define more effective antigens, whereas systems biology will increase our understanding of how changes in the expression of specific genes correlate with protective immune responses[1–4]. In turn, this will enhance our understanding of how to induce specific immune responses and, thus, the development of new vaccines. We suspect that systems biology will be most useful field of study for explaining the action of adjuvants (BOX 1) — knowledge that will allow us to choose the right ones to use in different circumstances — and for elucidating the ways in which to induce different T cell subsets to influence protection against infection, carriage and disease.

The future

Without doubt, the agents for which we need new vaccines are more complicated in their pathogenesis than those for which we have vaccines already, and we therefore need a more profound knowledge of the immune system than we have currently. This is particularly important for diseases for which natural immunity is absent or imperfect, such as HIV/AIDS and malaria. Nevertheless, the past has been a prologue to that hoped-for future, and all of the recently developed vaccines have been based on a preceding analysis of the protective immune response. No vaccine since the *M. bovis* bacille Calmette–Guérin vaccine has been developed without an immunological hypothesis about protection.

Hope for the future does not require denigration of the past. Although, as Abraham Lincoln said in his Annual Message to Congress, 1 December 1862, "the dogmas of the quiet past are inadequate to the stormy present", much has been accomplished using classical immunology. Even in the future, it is likely that the success of vaccines will depend on the induction of functional antibody that will prevent the acquisition of infection, and on specific cellular functions that control pathogen replication if infection occurs despite the presence of antibody[79]. These concepts are old; what is new is our ability to design antigens, to invoke the innate immune system in order to enhance adaptive immunity, and to characterize the T cells that are needed for the responses we want (BOX 2).

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Competing interests statement
The authors declare no competing financial interests.