

# History of vaccination

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**Vaccines have a history that started late in the 18th century. From the late 19th century, vaccines could be developed in the laboratory. However, in the 20th century, it became possible to develop vaccines based on immunologic markers. In the 21st century, molecular biology permits vaccine development that was not possible before.**

killed vaccines | proteins | live vaccine | genetic engineering

One of the brightest chapters in the history of science is the impact of vaccines on human longevity and health. Over 300 y have elapsed since the first vaccine was discovered. In a short article, it is not possible to do justice to a subject that encompasses immunology, molecular biology, and public health, but several more extensive sources are available to the interested reader (1–5). Rather than attempting a chronological narrative, I will consider vaccine development from the viewpoint of the technologies used to create vaccines. However, Table 1 provides a general idea of the chronology.

In current articles that describe novel technologies, it is often said that they will enable “rational” development of vaccines. The opposite of rational is irrational, but presumably the writers mean to contrast rational with “empiric.” However, in fact, vaccine development has been based on rational choices ever since the mid-20th century, when immunology advanced to the point of distinguishing protection mediated by antibody and that mediated by lymphocytes, and when passage in cell culture permitted the selection of attenuated mutants. After that point, successful vaccines have been “rationally” developed by protection studies in animals; by inference from immune responses shown to protect against repeated natural infection (the so-called mechanistic correlates of protection) (6); and from the use of passive administration of antibodies against specific antigens to show that those antigens should be included in vaccines.

## Attenuation

The idea of attenuation of virulent infections developed slowly over the course of centuries. Variolation was analogous to the use of small amounts of poison to render one immune to toxic effects. Jenner’s use of an animal poxvirus (probably horsepox) to prevent smallpox was essentially based on the idea that an agent virulent for animals might be attenuated

in humans (7). This idea played a role in the development of bacillus Calmette–Guérin but is even more obvious in the selection of rhesus and bovine rotavirus strains to aid the creation of human rotavirus vaccines as mentioned below under *Reassortment*.

It was Pasteur and his colleagues who most clearly formulated the idea of attenuation and demonstrated its utility, first with *Pasteurella multocida*, the cause of a diarrheal disease in chickens (8), then anthrax in sheep and most sensationally rabies virus in animals and humans (9). Their first approaches involved exposure to oxygen or heat, both of which played a role in the development of the rabies vaccine and in the famous anthrax challenge experiment at Pouilly-le-Fort (10). However, the more powerful technique of serial cultivation of a pathogen in vitro or in inhuman hosts originated with Calmette and Guérin, who passaged bovine tuberculosis bacteria 230 times in artificial media to obtain an attenuated strain to protect against human tuberculosis (11). Later in the 20th century, Sellards and Laigret (12) and, more successfully, Theiler and Smith (13) attenuated yellow fever virus by serial passage in mice and in chicken embryo tissues, respectively.

## Cell Culture

By the 1940s, virologists understood that attenuation could be achieved by passage in abnormal hosts. Notably, Hilary Koprowski and coworkers developed rabies and oral polio vaccines by passage in chicken embryo or mice (14, 15). However, this method was inefficient, and mice were not a sterile medium. A revolution happened with the discovery that cells could be cultured in vitro and used as substrates for viral growth. Enders, Weller, and Robbins (16) showed that many viruses could be grown in cell culture, including polio and measles, and this method was vigorously taken up by vaccine developers. The oral polio vaccine of Albert Sabin and the measles, rubella, mumps, and

varicella vaccines were all made possible through selection of clones by cell-culture passage in vitro (17–21). In essence, passage in cell culture leads to adaptation to growth in that medium, and the mutants best capable of growth have often lost or modified the genes that allow them to infect and spread within a human host. The oral polio vaccine is a good example, in that the mutants that occur in cell-culture passage that confer inability to cause paralysis were isolated by selection of clones with low neurovirulence in monkeys. These mutations are at least partly lost after replication of attenuated strains in the human intestine, leading to rare cases of paralysis after vaccination (22). Adaptation of viruses to growth at temperatures below 37 °C, the normal temperature of humans, also is attenuating, as was the case for rubella vaccine (20). Another live vaccine, thus far used only in the military to prevent epidemic pneumonia, consists of adeno 4 and 7 viruses grown in human diploid cell strains and administered orally to replicate in the intestine (23). Other live vaccines attenuated in cell-culture passage are the monovalent rotavirus vaccine attenuated by passage in Vero cells (24) and the Japanese encephalitis strain SA14-14-2 (25).

## Reassortment

Certain RNA viruses have segmented genomes that can be manipulated in a way similar to the chromosomes of eukaryotes. Cocultivation of two viruses in cell culture with clone selection by plaque formation allows isolation of viruses with RNA segments from both viruses. Reassortment has

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**Table 1. Outline of the development of human vaccines**

Live attenuated	Killed whole organisms	Purified proteins or polysaccharides	Genetically engineered
<b>18th Century</b>			
Smallpox (1798)			
<b>19th Century</b>			
Rabies (1885)	Typhoid (1896) Cholera (1896) Plague (1897)		
<b>Early 20th Century, first half</b>			
Tuberculosis (bacille Calmette–Guérin) (1927) Yellow fever (1935)	Pertussis (1926)  Influenza (1936) <i>Rickettsia</i> (1938)	Diphtheria toxoid (1923)  Tetanus toxoid (1926)	
<b>20th Century, second half</b>			
Polio (oral) (1963)	Polio (injected) (1955)	Anthrax secreted proteins (1970)	Hepatitis B surface antigen recombinant (1986)
Measles (1963) Mumps (1967)	Rabies (cell culture) (1980) Japanese encephalitis (mouse brain) (1992)	Meningococcus polysaccharide (1974) Pneumococcus polysaccharide (1977)	Lyme OspA (1998) Cholera (recombinant toxin B) (1993)
Rubella (1969)	Tick-borne encephalitis (1981)	<i>Haemophilus influenzae</i> type B polysaccharide (1985) <i>H. influenzae</i> type b conjugate (1987)	
Adenovirus (1980) Typhoid ( <i>Salmonella</i> TY21a) (1989) Varicella (1995)	Hepatitis A (1996) Cholera (WC-rBS) (1991) Meningococcal conjugate (group C) (1999)	Typhoid (Vi) polysaccharide (1994) Acellular pertussis (1996)	
Rotavirus reassortants (1999) Cholera (attenuated) (1994) Cold-adapted influenza (1999)		Hepatitis B (plasma derived) (1981)	
<b>21st Century</b>			
Rotavirus (attenuated and new reassortants) (2006) Zoster (2006)	Japanese encephalitis (2009) (Vero cell) Cholera (WC only) (2009)	Pneumococcal conjugates* (heptavalent) (2000) Meningococcal conjugates* (quadrivalent) (2005) Pneumococcal conjugates* (13-valent) (2010)	Human papillomavirus recombinant (quadrivalent) (2006) Human papillomavirus recombinant (bivalent) (2009) Meningococcal group B proteins (2013)

\*Capsular polysaccharide conjugated to carrier proteins.

enabled the creation of three major vaccines: live and inactivated influenza (26, 27), as well as one of the two rotavirus vaccines (28). In the case of inactivated influenza, the objective is to select the segments coding for hemagglutinin and neuraminidase and to combine them with segments coding for the internal genes of viruses that grow well. Thus, one obtains a vaccine virus that is safe to handle but still generates functional antibodies against virulent influenza strains.

In the case of live influenza vaccine, the hemagglutinin and neuraminidase RNA segments were reassorted with a previously attenuated cold-adapted virus. More recently, reverse genetics has been used to generate the attenuated strains (29).

Reassortment has also been used to make rotavirus vaccines. The first, developed by Kapikian et al. (30), consisted of one animal rotavirus and three reassortants, each containing 10 RNA segments from a rhesus

rotavirus and one coding for the VP7 surface protein of human rotaviruses (30). Because of safety issues, that vaccine was superseded by a pentavalent vaccine combining RNA segments from a bovine rotavirus with one segment from human rotaviruses coding for either surface V44 or VP7 proteins, (31) as well as the monovalent vaccine previously mentioned under *Cell Culture*.

### Inactivation

Another discovery toward the end of the 19th century was that immunogenicity could be retained if bacteria were carefully killed by heat or chemical treatment. The first inactivated vaccines were developed more or less simultaneously by Salmon and Smith in the United States and the Pasteur Institute group (Roux and Chamberland) in France (32, 33). Inactivation was first applied to pathogens such as the typhoid, plague, and cholera bacilli. This era was marked by competition bet-

ween French, German, and English workers to develop antibacterial vaccines. Inactivated vaccines against typhoid were first applied by Wright and Semple in England and Pfeiffer and Kolle in Germany (34, 35). Humans were vaccinated against plague by Haffkine, using inactivated plague bacilli (36). Live vaccines against cholera were developed by Ferran in Spain and Haffkine in France (37), but it was ultimately the vaccine developed by Kolle using heat-inactivated cholera bacilli that came into general use (38). That vaccine was given parenterally but was painful and did not give long-lasting immunity. More recently, a vaccine was developed that consists of orally administered killed cholera bacteria, with or without the B subunit of cholera toxin (39). Formalin-inactivated whole-cell pertussis vaccine was first tested by Madsen (40) and was later shown to be relatively successful in controlling serious disease (41). However, it was the later work of Kendrick and Eldering that

permitted standardization and safety of a whole-cell vaccine (42).

In 1923, Glenny and Hopkins made diphtheria toxin less toxic by formalin treatment (43). Ramon improved on this discovery and showed it was possible to inactivate the toxicity of those molecules yet retain their ability to induce toxin-neutralizing antibodies (44).

In the 20th century, chemical inactivation was also applied to viruses. Influenza vaccine was the first successful inactivated virus vaccine (45), and experience with that vaccine served Salk well in his successful effort to develop an inactivated polio vaccine (46). Later, hepatitis A vaccine was prepared by Provost and coworkers, also based on chemical inactivation (47). The excellent efficacy of the latter testifies to the ability of careful inactivation to maintain immunogenicity.

Whole inactivated viruses or subunits of virus have been used to make successful vaccines against Japanese encephalitis virus and tick-borne encephalitis virus (48–50).

### Capsular Polysaccharides

Early in the history of bacteriology, morphological studies and chemical analysis showed that many pathogens were surrounded by a polysaccharide capsule and that antibodies against the capsule could promote phagocytosis. The first use of this information to make a vaccine was the development of meningococcal polysaccharide vaccine by Artenstein, Gottschlich, and coworkers (51). This vaccine controlled epidemic and endemic disease in military recruits. Basic bacteriology also suggested that pneumococcal polysaccharides were immunogenic although there were chemical differences between the multiple serotypes. Heidelberg and Macleod and later Austrian fostered the creation of combinations of multiple pneumococcal polysaccharides to prevent invasive infections (52, 53). This principle was then applied to *Hemophilus influenzae type b* capsular polysaccharide by Anderson, Smith, Schneerson, Robbins, and coworkers (54, 55). The Vi antigen present in the capsule of the typhoid bacillus was made into a vaccine by Landy and coworkers (56).

All of the capsular polysaccharide vaccines generated serum antibodies that prevented bacteremia and thus end-organ disease in adults, but they were not immunogenic in infants, who are unable to mount a B-cell response to polysaccharide alone. This problem was solved by coupling the polysaccharides to proteins, which allowed T-cell help to B cells. In addition, whereas the polysaccharide vaccines did not prevent nasopharyngeal carriage of the bacilli, conjugated vaccines did prevent carriage and thus added the dimension of herd immunity to immunization

against the three major bacterial pathogens of infancy. Curiously, the utility of protein conjugation of polysaccharides had been shown by Avery and Goebel in 1929 (57), but this discovery was not taken advantage of until Schneerson, Robbins, and coworkers made a conjugated *H. influenzae type b* vaccine (55). Eventually, this principle was applied to meningococcal and pneumococcal vaccines, with resulting control of both invasive infections and spread of the organisms. Hib and some meningococcal serogroups have been completely controlled whereas pneumococcal serogroups in vaccines have greatly diminished disease causation.

### Protein-Based Vaccines

Aside from tetanus and diphtheria toxoids, mentioned above under *Inactivation*, several vaccines consist of partly or fully purified proteins. Most inactivated influenza vaccines used today are generated by growing the viruses in embryonated eggs and then breaking up the whole virus with detergents. The viral hemagglutinin (HA) protein is purified to serve as the vaccine antigen although other components of the influenza virus may be present in the final product (58).

Acellular pertussis vaccines have replaced whole-cell pertussis vaccines in many countries to reduce reactions to the latter. The licensed acellular vaccines consist of one to five proteins from the pertussis bacillus, which are meant to reconstitute efficacy of the whole-cell vaccine without generating febrile reactions. Sato and Sato created the first such vaccine for use in Japan in 1981 (59), but many other acellular vaccines were licensed after extensive trials conducted in the 1990s (60).

Although Pasteur and coworkers made inactivated whole-cell anthrax vaccine early in the history of vaccinology, it was only in the early 1960s that a vaccine was developed for biodefense by the US Army, based on anthrax protective antigen protein secreted by the organism (61). Another improvement on a vaccine originally developed by Pasteur was the creation of a cell culture-produced rabies vaccine by Wiktor, Koprowski, and coworkers in the 1970s (62). Human, monkey, or chicken cells are used to grow the virus, which is then purified and inactivated. The rabies glycoprotein is the protective antigen in the vaccine.

### Genetic Engineering

The revolution of genetic engineering toward the end of the 20th century has greatly impacted vaccine development. The first fruit of that revolution was the vaccine against hepatitis B. Initially, Hilleman and coworkers had purified the hepatitis B surface antigen particles from the serum of naturally infected

patients and inactivated any residual live virus (63). However, this type of vaccine could not be practical in the long term. Valenzuela et al. (64) placed the coding sequence for the S antigen into yeast cells and were able to produce large quantities of surface-antigen particles in vitro. Genetic engineering has been used to produce many candidate antigens for vaccines in yeast, animal cells, or insect cells producing an antigen in culture.

Two bacterial live-virus vaccines are administered orally: the Ty21a vaccine against typhoid, which is a strain mutated chemically to deprive the organism of enzymes that contribute to virulence (65), and the CVD103-HgR cholera vaccine, which is unable to synthesize complete cholera toxin (66). Both of these vaccines were made possible after genetic engineering provided the tools for excision of bacterial DNA.

Many viruses and bacteria are under active study as vectors for vaccine antigens. Poxviruses, adenoviruses, bacillus Calmette–Guérin, and other relatively attenuated microbes have had genes for protective antigens from pathogens inserted into their genomes. The vectors are then injected and undergo either abortive or complete replication, expressing the inserted genes in both cases. The first licensed vector is the 17D yellow fever attenuated strain, which serves as a vector for the prM and E genes of Japanese encephalitis virus, thus immunizing against the latter (67).

The development of the human papilloma virus (HPV) vaccine was made possible because of the properties of the L1 protein of the virus (68, 69). This protein induces protective antibodies, but what makes it particularly immunogenic is that it aggregates to form virus-like particles (VLPs) that are much more immunogenic than the soluble protein. L1 is produced in yeast or insect cells, and the VLPs produced therein form the basis of the current vaccines.

Influenza HA has been produced in insect cells and induces antibodies without the risk of allergy to egg proteins (70, 71).

A vaccine against Lyme disease was on the market briefly. The vaccine consisted of the OspA protein of *Borrelia burgdorferi*, produced in *Escherichia coli* (72, 73).

Most recently, a meningococcal group B vaccine has been licensed, consisting of four proteins identified by genomic analysis that induce bactericidal antibodies together with an outer membrane vesicle of the organism (74). This is the first vaccine developed by so-called reverse vaccinology, pioneered by Rappuoli and coworkers (75), by which genomic analysis enables selection of proteins that induce protective immune responses.

## The Past is Prologue

Many have pointed out that it is easier to foretell the past than the future! Be that as it may, the current tendencies in vaccine development are reasonably clear. Although the older methods described above continue to be used, as for example inactivation of whole virus to make vaccines against enterovirus 71 (76), expression of proteins by transcription and translation from either DNA or RNA coding for those proteins will be a widely used approach (77, 78). Attenuated viral or bacterial vectors carrying genetic information for a foreign vaccine antigen is a prominent

strategy, exemplified by candidate HIV and dengue vaccines (79, 80). As described above, replicating organisms often make good vaccines, but ways are available to allow only one replication cycle to produce so-called replication-defective agents that maximize safety (81). To generate higher immune responses, stronger adjuvants than aluminum salts are coming into use, including oil-in-water preparations and Toll-like receptor agonists, and their use will surely increase (82).

Meanwhile, structural biology and systems biology are enabling us to identify critical protective antigens and the immune responses

they generate, including those that are innate (83, 84). Major unsolved problems remain, including how to deal with immaturity and postmaturity of immune responses in the young and old, respectively; how to induce mucosal responses with nonliving antigens; how to prolong immune memory; and genetic variability as it affects both the safety and efficacy of vaccines. Future vaccines are likely to have a more complex composition than heretofore, but the principles elucidated by past successes will have continued importance as vaccination is extended to more diseases and to all age groups.

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