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ETHICAL ISSUES IN VACCINE RESEARCH AND DEVELOPMENT

Abstract: The early development of the concept of immunization and the first vaccines was based mostly on courageous work of visionaries such as Edward Jenner, Louis Pasteur and Robert Koch. From those first attempts of vaccination in the 18th century, the development of vaccines was further encompassed by some of the most significant achievements in the field of immunology, molecular biology and genetics. The development of vaccines changed the picture of global health. As the most life-saving innovation in the history of medicine, vaccines have eradicated some diseases, reduced the mortality of others and prevented many types of disabilities. From the late 18th century to modern innovative, cutting-edge technologies in the vaccine research, thousands and thousands of researchers, some who caught the spotlight and some who remained anonymous within the walls of their laboratories, contributed to their safety and efficacy.

The key ethical debates surrounding the vaccines revolve around several questions, such as mandatory vs. voluntary vaccination, the ethics of vaccine development and testing, informed consent regarding risks and benefits of the vaccination, and the disparities in distribution and availability. In more developed countries, the ethical issues regarding vaccination tend to focus on the rights of individuals vs. regulatory bodies. Those issues, together with the growing anti-vaccination movement and vaccine-hesitancy, have become more significant during the time of COVID-19 pandemics.

This narrative review gives a summary of the most important breakthroughs in the history of vaccine development, but also focuses on the emerging problems regarding ethics and controversies surrounding the issue of vaccination during catastrophic pandemics that affected the world.

Keywords: vaccine, infectious diseases, epidemics, development
Non MeSH: Ethics

Research in vaccine development: from empirical to rational

The foundation of the modern concept of vaccination was laid in 1796 by Dr Edward Jenner. Jenner was an English physician and a researcher who transferred his observation that milkmaids who had been infected with cowpox were immune to outbreaks of smallpox, into the one of the world's first experiments roughly resembling clinical trials. Cowpox is similar to but much milder than the highly contagious and often deadly smallpox disease. The first subject in this chilling experiment was an eight-year old boy, followed by 22 subsequent participants. [1] The boy had some of the pus from cowpox lesions of a young milkmaid scratched into his skin by Dr Jenner. This and the subsequent experiments were considered successful since the subjects became immune to smallpox after being inoculated with it six weeks after exposure to fresh cowpox lesion material. The Latin word for cow is *vacca* and cowpox is *vaccinia* therefore Jenner decided to call this new procedure "vaccination". Edward Jenner privately published the results of his research of 23 cases in a 1798 monograph, "An inquiry into the causes and effects of the Variolae Vaccinae, a disease discovered in some of the western counties of England, particularly Gloucestershire, and known by the name of the cow-pox", after his attempt to publish a short communication on this topic was rejected by the Royal Society. Although unacceptable by contemporary bioethical standards, Jenner's experiments not only replaced the high-risk, previously adopted practice of "variolation", the African, Indian and Chinese method of inoculating with low doses of smallpox (the pulvis from dried scabs) in hopes of building up the immunity to the disease, his method that underwent biotechnological changes over the next two centuries, eventually resulted in the eradication of this devastating disease in 1979. [2]

Louis Pasteur's 1885 vaccine against rabies, an unavoidably fatal disease with dramatic symptoms such as hydrophobia, paranoia, terror, mania, hallucinations, and delirium, was the next to make a tremendous impact on public health. [3] And then, with the development of microbiology, vaccines and antitoxins against many infectious diseases were introduced throughout the 1930s.

Since those early steps in the development of vaccinology, enormous leaps forward have been made. The iron lungs and braces designed for the victims of poliomyelitis, the disfigurements left in rare survivors of smallpox, the distinguishing sounds of the whooping cough, the barking cough associated with the diphtheritic croup, the scary opisthotonos in tetanus are nowadays either never or very rarely seen, at least in developed countries. Some of the scourges of humanity such as smallpox, measles or rabies are either eradicated or under control. The disease targets in modern vaccinology have expanded, and a significant part of the research is now aimed at the prevention of non-infectious diseases, such as cancer or allergies.

The development of the vaccines from the viewpoint of biotechnology is encompassed by some of the most significant achievements in the field of immunology, molecular biology and genetics. Since the early attempts of variolation (the use of small amounts of virulent material to render immune response), the first step forward in terms of attenuation of the virulent agent in order to provide safer ways of develop-

ing immunity was Jenner's idea of using the virus passed through another host, a live animal in this case, which weakened the virus. The weaker, less virulent viruses were more easily conquered by the immune system, leading to long-term protection. Although unaware at the time, Jenner had laid the foundation for what would later become live, attenuated vaccines. The process of attenuation was scientifically formulated by Louis Pasteur. His first approach involved culturing the microbes exposed to atmospheric oxygen for prolonged culture intervals, which played a role in the development of the vaccine against anthrax. At the same time, Pasteur's main competitor, Henri Toussaint, a professor of Anatomy and Physiology at the Veterinary School of Toulouse, generated his anthrax vaccine simply by killing the bacteria by heating for 10 min at 55°C and in later experiments by subjecting them to the action of carbolic acid or potassium-bichromate in the process of chemical attenuation. [4].

Pasteur contested the findings of Toussaint because he allegedly did not believe in the chemical method and the rivalry led to the famous anthrax challenge experiment at Pouilly-le-Fort in 1881 where Pasteur successfully demonstrated his concept of vaccination in sheep. However, Dr Robert Koch heavily challenged and objected Pasteur's claim of having discovered the process of poisons modification (e.g. microbial attenuation) and refused to recognize the value of Pasteur's attenuation method with both practical and theoretical implications. Koch also stated that the biological and chemical characteristics of a microbials were not only specific but also permanent. [5]). The real nature of the Pasteur's anthrax vaccine in this famous experiment remained unknown. According to Pasteur's recent biography by Gerald L. Geison, Pasteur secretly used the vaccine of Toussaint prepared by the treatment with potassium-bichromate. [6] However, an earlier biography by Vallery-Raddot had already recognized Toussaint's priority in developing an anthrax vaccine and the fact that Pasteur simply reproduced his experiments. [7] Despite all the controversies and ethical issues, including personal relations between the two scientists, the development of the first artificially attenuated vaccine revolutionized the prevention in infectious diseases and Pasteur gave these artificially weakened products the generic name "vaccines", in honor of Jenner's groundbreaking discovery. [7]

It is now generally accepted that Edward Jenner invented vaccination and Louis Pasteur invented vaccines. [8] The powerful technique of serial cultivation of a pathogen in vitro or in in habitual hosts was developed by Calmette and Guérin, two French scientists whose names are enshrined in the well-known abbreviation BCG (Bacillus Calmette-Guérin). [9, 10] Albert Calmette and Camille Guérin had been working on developing a vaccine against tuberculosis since 1905. Tuberculosis is still a serious medical threat worldwide, despite the availability of antituberculotic medications and a vaccine. Bacillus Calmette-Guérin (BCG), the tuberculosis vaccine, is an attenuated mutant of Mycobacterium bovis, the causative agent of tuberculosis in cattle which is related to Mycobacterium tuberculosis, the bacillus which causes human tuberculosis. [11]. Drs Calmette and Guérin initially hypothesized that a bovine tuberculosis bacillus could transmit pulmonary tuberculosis after oral administration, however after 39th passage through ox bile medium, the strain lost its lethal effect to experimental animals [11, 12]. Calmette and Guérin subcultured bovine tuberculosis bacteria 230

times in this media and actually obtained an attenuated strain which had the potential to protect against human tuberculosis and, in 1921, the BCG vaccine was finalized. [9]

That same year, it was first used in a human when it was given to a baby of a mother deceased due to tuberculosis, at the Charité Hospital in Paris, by Dr Weil-Hale. [13]. The child had no adverse reactions. Dissemination of BCG around the world began in 1924 and different methods of subculturing were developed. It is estimated that approximately 3 billion doses of BCG vaccine have been used worldwide to protect the population against tuberculosis, yet the mechanism that causes the attenuation of BCG is still poorly understood. [11]. BCG contains not a unique strain of the bacillus, but numerous genotypically and phenotypically different substrains. We are just beginning to understand the molecular mechanisms beneath the immunogenic properties of this vaccine. It is remarkable that the current BCG strains comprise natural mutants of well-recognized virulence factors, among which the loss of secreted lytic function required for invasion of lung interstitial tissue may be the main mechanism of attenuation. [11, 14]The method of attenuation by passage through live animal or animal derived tissue was further developed later in the 20th century by Sellards and Laigret and Theiler and Smith who attenuated yellow fever virus by subculturing in mice and in chicken embryo tissues, respectively. [9] Some of the most common vaccines still used, including measles and mumps basically use this approach. [9, 15]Another early and very effective empirical approach in the development of vaccines based on whole pathogens was inactivation. The method is grounded on the concept that pathogen microorganisms preserve immunogenicity when carefully killed by thermal or chemical treatment. The first inactivated vaccines were developed almost simultaneously two teams, the one in the United States led by Salmon and Smith and the other at Pasteur Institute in France, led by Roux and Chamberland. [9]

Inactivation was first applied to pathogens such as the typhoid, plague, and cholera bacillus, nowadays most used inactivated vaccines are polio vaccine and the seasonal influenza vaccine (in the form of injection). The advantage of inactivated vaccines is the inability of the pathogen to revert to a more virulent form capable of causing the disease, which is a rare possibility with live attenuated vaccines, but the downside is that they tend to provide a shorter length of duration of protection compared to attenuated vaccines, and are more likely to require boosters to create long-term immunity. [16]One of the most famous scientific rivalries of the 20th century actually revolved around the inactivated vs. attenuated principle of vaccine creation. Poliomyelitis, commonly shortened to polio, is a highly infectious viral disease that used to affect thousands of children, mostly under 5 years of age, but the older children and even adults were not spared. The poliovirus can invade the nervous system and cause paralysis. Of the three wild strains of the poliovirus, type 2 was eradicated in 1999, the latest cases of type 3 were reported in 2012 and as in 2020 only type 1 affects some Asian and African countries. [17] There are to scientists to credit for dramatically reducing the incidence of this terrifying disease: Drs Albert Sabin and Jonas Salk and the controversies surrounding their contribution in conquering polio outlived both of them. Sabin was nearly ten years older than Salk and the two clashed from the beginning. The first approved polio vaccine was an inactivated one. It was created by Salk using virus

grown on monkey kidney cells and inactivated with formalin. In 1954, this vaccine was tested in a placebo-controlled trial, which enrolled 1.6 million children from the United States, Canada and Finland, with immediate success. [17] However, Salk's inactivated vaccine had some downsides: the titre of the circulating antibodies would decrease within a few years after vaccination, the production required use of a large number of monkeys and, after licensing, some manufacturers failed to adequately inactivate the virus with devastating consequences. Although Salk's was the first polio vaccine, it was not to be the last; in the meantime, a live-virus vaccine for polio was being developed by Albert Sabin. Sabin, like many scientists at that ime, believed that only a living virus would be able to guarantee immunity for a prolonged period. [18]

Albert Sabin introduced an oral attenuated vaccine in the 1960s. In 1960 Sabin published in JAMA results obtained with his newly developed trivalent oral vaccine administered to 26033 children in South America. The strains developed by Sabin provided good antibody levels and were less neurotropic for monkeys, and Sabin's live vaccine attenuated oral polio vaccine has seemingly won the race for supremacy in the fight against polio. [19] The live attenuated vaccine is administered orally, so it eliminated the need for trained staff and sterile syringes and was suitable in mass campaigns. However, the live attenuated oral polio vaccine is related to certain adverse effects, such as the cases of Vaccine-Associated Paralytic Poliomyelitis (VAPP) and the emergence of mutated strains of poliovirus. After nearly forty years, at the very beginning of the 21st century, most developed countries switched the schedule of vaccination against polio by using the modern inactivated intramuscular polio vaccine instead of the oral, live vaccine, since the two have similar efficacy, but the inactivated one is now considered safer. The disadvantages of the worldwide introduction of the inactivated vaccine are its cost, the intramuscular administration, its inability to produce adequate intestinal immunity and the demanding manufacturing process. [20] As a kind of a poetic reconciliation between the two concepts and the two great scientists, the World Health Organization under its plan for the polio-free world recommends the combined use of the novel (bivalent) live attenuated oral polio vaccine in combination with the at least one dose of the inactivated intramuscular vaccine as the most efficient way towards eradication of this disease throughout the remaining polio endemic countries. [17]

Although a significant proportion of currently manufactured vaccines were developed empirically through an "isolate, inactivate or attenuate, and administer" approach, not all infectious diseases could have been conquered using this method. [21] Safety concerns were also associated with conventional vaccine preparations based on whole pathogens such as inactivated or attenuated bacteria or viruses. [22] The conventional approach in vaccines development failed for pathogens such as HIV, which have an array al of molecular tricks to avoid immune responses. In such cases, alternate strategies based on rational approach are being investigated. [23]

Despite very advanced knowledge of immune mechanisms involved, the role of specific of elements of the immune system in this process is still unclear for the majority of pathogens. [22] The immune system is highly complex and the effects of coordinated activity of its parts are usually not equal to the sum of parts. Therefore, the uni-

versal strategy for the rational design of vaccines does not exist. The selection of the immunogenic part of the pathogen, the pathogens' mechanisms of evasion, the longevity of the immunity and the identifications of markers of vaccine efficacy or adverse reactions are all still unpredictable and that is the reason why even with the development of the sophisticated methods within the rational approach, the data from the empirically developed vaccines are still of a tremendous value.

Rationally designed vaccines comprise composed antigens (molecules capable of stimulating an immune response), delivery systems and sometimes adjuvants that elicit predictable immune responses against specific epitopes to protect against defined pathogen. [22] Epitope is an antigenic determinant, actually the part of an antigen recognized by the immune system, specifically by antibodies, B cells, or T cells. More precisely, the epitope is a specific piece of antigen to which an antibody can bind. Novel technologies such as vaccine conjugation (covalent linkage of bacterial polysaccharides to carrier proteins) and the introduction of modern vaccine adjuvants (agent that increases specific immune responses to an antigen) changed the field of vaccinology. However, the breakthrough came with the sequencing of the *Heamophilus influenzae* whole genome in 1995, a moment described as the birth of "Reverse Vaccinology," a novel, genome-based approach to vaccine development. [23, 24] This huge step forward towards rational vaccine development allowed throughput screening and profiling to determine relevant antigens which contain peptides that can be synthetized and manufactured at relatively cost and incorporated in the so-called subunit vaccines. Identification of the candidate antigen and understanding its structure enables rational design to fine tune its presentation to the immune system and to facilitate its manufacturing. This approach, if applicable, allows relatively fast production of large quantities of vaccines, which is of extreme importance during epidemics. The high purity of the subunit vaccines is their advantage due to adverse reactions risk reductions. The subunit vaccines, however, lack some molecules needed for the stimulation of the early, innate immune response. This hurdle is usually overcome with the use of an adjuvant. The adjuvants increase and modulate the immune response. Aluminum salts were the only adjuvants in use until 1990's when some other vaccines were licensed using novel adjuvants. The most common subunit vaccines are those against certain viral diseases and include the subunit vaccine against hepatitis B virus that is composed of only the surface proteins of the virus, the vaccine against human papilloma virus (HPV) that is composed of the viral major capsid protein and the hemagglutinin and neuraminidase subunits of the influenza virus. Most of these also contain adjuvants.

Among the different approaches to the design of the vaccines, conjugated polysaccharide vaccines have been proven reliable and cost-effective in the prevention of many bacterial diseases such as Haemophilus influenzae type b (Hib), Streptococcus pneumoniae, Neisseria meningitidis and Salmonella Typhi. The process of conjugation is achieved by covalent linkage of bacterial polysaccharides to carrier proteins, and have been demonstrated to overcome the limitations frequently imposed by the use of poorly immunogenic unconjugated polysaccharide vaccines. [24]Antigen delivery systems became important when antigens cannot be efficiently transported and presented to the immune system. [22] Different recombinant or attenuated viruses have

been investigated in order to overcome this problem. Viral vector-based vaccines present advantages in terms of induction robust immune response without an adjuvant and enhance a broad range of immunogenicity without an adjuvant and are suitable for the "prime-boost" immunization strategy. This type of prime-boost immunization means administration of two different vectors or delivery systems expressing the same or similar antigens. This has been known to greatly increase both antibody and T cell immunogenicity when performed using certain vector combinations and is one of the strategies currently employed in the development of the vaccine against SARS-CoV-2 and previously in Ebola vaccine trials. Currently, several vaccine candidates for COVID-19 are based on this approach. These vaccines use viral vectors (mostly attenuated human adenovirus) which carry the spike gene of the target virus, replicate and cause the immune response. One of the possible disadvantages of this type of vaccines is that the prior exposure to the vector virus may reduce the immunogenicity of the vaccine. In the case of adenoviruses used as vectors in the COVID-19 vaccine development, the major downside is that adenoviruses circulate widely, causing the common cold, and some people harbor antibodies that will target the vaccine, making it ineffective. This obstacle may be solved by using primate adenoviruses. Chimp adenoviruses were investigated for HIV and malaria at the University of Oxford. According to Oxford research, the use of the attenuated primate adenovirus in a COVID-19 vaccine will make it ineligible for the potential malaria vaccine, because those vaccinated for the coronavirus will have antibodies against the vector. [25].

One of the very promising cutting-edge technologies is the nucleic acid (DNA and RNA) vaccine design for protection against not only infectious, but also malignant diseases. [26] These vaccines come in various forms. The technique is based on injecting genetically engineered DNA (as a plasmid) or RNA (as mRNA). Plasmid DNA vaccines have shown great promise in animal models but are not sufficiently potent in humans. Another problem, related to all gene therapy, is that the DNA may be integrated into the host chromosome, resulting in oncogenes activation or the turn off of tumor suppressor genes. The mRNA vaccines represent a promising alternative to conventional vaccine approaches. [27] At the moment, the forerunner in the dramatic race for a safe and efficacious COVID-19 vaccine relies on mRNA technology which has its relative merits and limitations. It was developed by a relatively small company, at least by pharmaceutical industry standards, and after more than two hundred years after Jenner's work, two people were put in the spotlight again as recognizable individuals instead of an army of anonymous researchers. German Drs of Turkish origin, Ugur Sahin and Özlem Türeci, partners in professional and personal life, have dedicated their lives to the field of oncology and infectious diseases, and the years spent in research of personalized immunotherapy treatments of various diseases led to the couple's groundbreaking research and launched them into the public eye, as the brains behind the one of the world's first effective coronavirus vaccine. Their story is the example of shared passion and dedication, and also a reminder that, despite impressive technology worth billions of dollars invested by multinational pharmaceutical companies, behind every major breakthrough in science standing individuals with their ideas, knowledge, hard work and courage.

Ethical issues and vaccines

Vaccine research and development has always been accompanied not only by complex biotechnological challenges but also by pharmaco-economic and ethical issues. Traditionally, the return on the investment in vaccines was considered lower compared to other pharmaceutical products. Therefore, major pharmaceutical companies have more or less neglected this field for quite a long time. The key ethical debates surrounding the development and distribution of vaccines revolve around several key questions, such as mandatory vs. voluntary vaccination, the ethics of vaccine development and testing, informed consent regarding risks and benefits of the vaccination, and the disparities in distribution and availability. In more developed countries, the ethical issues that surround vaccination tend to focus on the rights of individuals vs. regulatory bodies. In low income, the fundamental issue is the lack of access to vaccines or high costs of the immunization. [28] These issues have been and still are thoroughly analyzed and discussed among many biomedical researchers, bioethicists, philosophers, lawyers, politicians, human rights activists and policy makers.

The general population's attitude towards vaccination, especially in more developed parts of the world has been gravely affected by the anti-vaccination movement. Although as old as the immunization itself, the recent rise in the opposition to vaccines in general, but in particular against the MMR (measles, mumps, and rubella) vaccine, can be attributed to the works of the disgraced British ex-physician, Andrew Wakefield and his, now retracted, article on the association of the MMR vaccine and autism published in The Lancet in 1998. [29] This has caused multiple measles outbreaks in Western countries where this disease was thought to be eradicated. [30] At the same time, the access to medical information of various qualities is now easier than ever, allowing misinterpretation or dissemination of wrongfully selected biased data. The social media probably play a crucial role in this process, by allowing uncontrolled dissemination of information and celebrities such as actors and music stars have taken roles of "experts", motivated sometimes by their personal, unfounded experiences or beliefs. [30] In the light of these events, the vaccines become a sort of victims of their own success. Many people who are now parents were born in the golden era of immunization, therefore undermining the fear and dangers associated with infectious diseases such as polio or diphtheria. But the landscape has recently rapidly changed. In the late twentieth century we believed that the infectious diseases had been mostly defeated and the field of infectiology was treated as the almost dying one. The epidemiologists turned to malignant and cardiovascular diseases. However, the infectious diseases returned with a vengeance. Malaria has never been more resistant to treatment, tuberculosis is showing its ugly face again, AIDS has changed the world since the 1980s, Ebola has claimed its many victims, measles are back as a result of the anti-vaccination movement and finally, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), also known as COVID-19, emerged late in 2019 and spread globally, catching the public health systems completely by surprise and off guard. We now witness the final moments of the one of the most exciting races in this field, with several COVID-19 vaccine candidates approaching the finish line which tremendous

hope and anxiety. It is now clear that we do not only face a devastating infectious disease outbreak, but also an "infodemic" of false information about COVID-19 and the spreading hesitance about the potential vaccine. [31]

Although every medical treatment and intervention isassociated with a certain degree of risk, it is the vaccine skepticism that carries the greatest risk to public health. The overestimation of the likelihood of negative events that is behind vaccine hesitancy and the risk/benefit ratio of vaccination, need to be carefully addressed, and the adequate education has to be provided to both, health care professionals and the general population.

Outlook

The development of vaccines changed the picture of global health. As the most life-saving innovation in the history of medicine, vaccines have eradicated some diseases, reduced the mortality of others and prevented many types of disabilities. From the early days of Jenner's experiments to contemporary innovative technologies in the vaccine research, thousands and thousands of researchers, some who caught the spotlight and some who remained anonymous within the walls of their laboratories, contributed to their safety and efficacy.

A pandemic that humbled the mankind showed us once again the importance of safe and efficient prevention and emphasizes the importance of ethics not only in biomedicine but also in media, journalism and digital environment. There are many ethical issues associated with the research in the field of vaccinology, but also in the vaccine manufacturing and distribution. Those issues have to be addressed with great caution. The vaccine skepticism, especially at this moment, presents a major public health threat.

COVID-19 caused the loss of nearly two million human lives so far. It exhausted even the most developed healthcare systems and seriously affected the global economy. It also taught us several important lessons. Evidence-based medicine is a powerful weapon. The unsanitary conditions are still a major public health concern in countries such as China and can still cause worldwide devastating consequences, such as the emergence of a novel virus. Therefore, providing reliable information on time vs. concealment of epidemiological data are of great importance. The common misconception that the investigation in the field of biomedicine, especially pharmaceutical, is driven by profit only is opposed with the fast and accurate reaction of many companies, including the major ones, who invested tremendously in the prevention of COVID-19. It also pointed out the dangers of populist leadership and the consequences of using the public health threat for gaining political power and influence. While discriminatory campaigns against migrants, were led by some governments, thereby against immigration in general, the success of Drs Sahin and Türeci, both born to parents from Turkey who moved to Germany in the 1960s, reminded of the precious (valuable?) human potential immigrants can bring to the welcoming and accepting societies.

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