Vaccines

History and Science

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Pathogenic agents

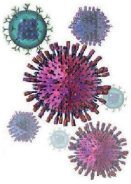
We are surrounded by microorganisms, such as bacteria, viruses, fungi or parasites, some of which can cause various diseases, so they are called ***pathogens***. Throughout the phylogenetic scale, animals have developed a series of defence mechanisms to defend themselves from pathogens. One of these mechanisms is the immune system, which attempts to mount a response against the foreign agent. The science that studies the immune system is called ***Immunology***.

Among the pathogens are **viruses**, which can only live in a host cell or in bacteria. Their minute size means that they have to be viewed using an electronic microscope. There is an enormous variety of viruses, and while there are viruses with a genetic content of ribonucleic acid (RNA), like the HIV virus that causes AIDS, others have deoxyribonucleic acid (DNA), like the Herpes simplex virus, some infect bacteria (bacteriophages), others only infect humans (smallpox virus). Viruses cause serious diseases in humans, most of which do not have an effective treatment.

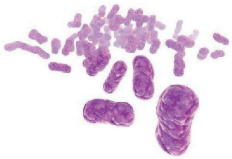
Parasites are also causal agents of numerous diseases, some of which, such as malaria, are very serious. There are a large variety of parasites, including *Leishmania, Giardia, Tripanosoma, Toxoplasma*, and *Entamoeba*, which cause numerous diseases throughout the world.

Microscopic **bacteria** can adopt various forms (bacillus, cocci and spirals), as well as different sizes, and cause a large number of infections, some of which can be lethal without appropriate treatment with antibiotics.

Fungi can cause diseases directly (called mycoses) or through their toxins. Some fungi, such as *Candida* or *Aspergillus*, cause diseases that can vary from the mild (vaginal infections) to the very serious (pneumonias, encephalitis), and can even lead to death.



Viruses



Bacteria

Fungi

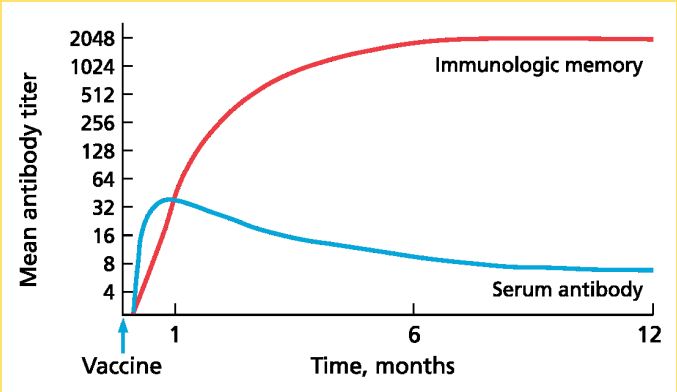
Parasites

Immune system

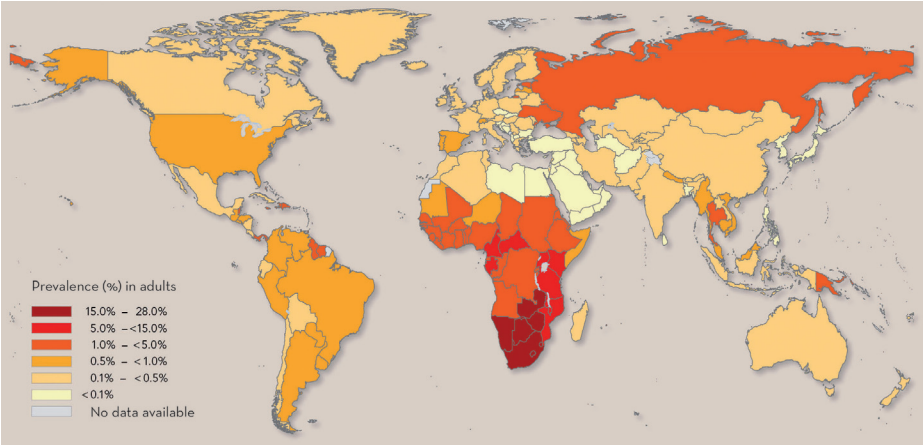
We have a group of cells and substances circulating in the lymphatic and blood vessels, as well as being located in certain tissues (the spleen, appendix, tonsils, lymph nodes, intestine), to respond against any foreign element that enters into our organism. The first cells to act after the entrance of a pathogen are the **macrophages and neutrophils**, which have the ability to capture the microorganisms by a process called phagocytosis. In addition to these and other sentinel cells, we have a more sophisticated recognition system, called the specific immune system. This includes two types of cells; **B and T lymphocytes**, which present specific receptors in their membrane and are activated and proliferate after recognizing foreign molecular structures. The activation of the B lymphocytes leads to production of antibodies (which will help in the destruction of the pathogen), whereas the T lymphocytes have different functions (cooperation, cytotoxicity or regulation) depending on the type of T lymphocyte.

A fundamental characteristic of these cells is that after being activated by a pathogen, they generate ***memory cells***, which can last throughout the life of an individual and can remember the same component to which they responded. This makes it possible for a person's immune system to mount a defence, when suffering an infection, for example the varicella (chickenpox) virus. Memory cells are then generated, which respond rapidly and efficiently, leaving the individual ***immunized*** against that pathogen, and only rarely will again suffer from the varicella virus.

Response kinetics after vaccination



Overview of HIV infection
33 millions of people lived with HIV in 2007



Resource: ONUSIDA – Joint United Nations Programme on HIV / AIDS

Given that we can have **immune memory**, and remember previous encounters with pathogens, it is possible to generate *vaccines*. The aim of vaccines is to activate the immune system in a specific way against a particular virus or bacterium, but **without producing the disease**. Therefore, vaccines are prepared with dead or attenuated microorganisms, fractions of the microorganisms or only with the toxins produced by them. After the vaccine is introduced into the organism, the immune system will react as if a real pathogen has entered, and will activate and generate memory lymphocytes and antibodies. Hence, the individual will remain protected, immunized when there is a virulent pathogen. Months or even years after a specific vaccination, when this particular pathogen appears, the person will already be protected, will act against the pathogen in a rapid and effective way, and will not then suffer infection. This is the principle on which **Vaccination/ Immunization** is based. However, with viruses that mutate regularly the memory lymphocytes are not useful, as they do not recognize the new forms of the pathogen. This means that it is necessary to design new vaccines (as each year for the influenza virus) or that the vaccines obtained are not effective (as with vaccines against the AIDS virus).

History of pathogens

The first written evidence about infectious diseases dates back to Egyptian papyrus documents (*The Babylonian Epic of Gilgamesh* 2000 BC), where there are descriptions of the illness and death of people from plagues. However, in that era, they were not associated with infectious agents, but were attributed to Punishments of the Gods.

In 430 BC, during an epidemic of plague in Athens (Greece), some very interesting observations were made, such as those of Thucydides, who stated that:

"Nobody suffers the disease twice, and if this occurs, the second attack is never fatal".

This sentence summarizes in an accurate and simple way two of the fundamental characteristics of the immune system of vertebrates: *memory* (remembering a previous exposure) and the *"maturing" or improvement of the response* (during a second exposure, the response is better). These two characteristics are going to be crucial for being able to make effective vaccines.

Together with the belief that plagues were sent by the anger of the gods, there was also the concept that diseases were caused by "ill humour" (an excess of one of the four humours, black bile, yellow bile, phlegm and blood), and this concept persisted for many centuries. This explains why doctors frequently carried out bleeding as therapy (on occasions with the death of the patient) with the aim of eliminating surplus blood, or purges for eliminating bile, or retained liquid. Moreover, they did not wash their hands and only rarely changed their clothes, as they did not know that bacteria or viruses could exist on their hands and clothing.

Until the discovery of pathogens (in the nineteenth century) and the arrival of the first antibiotics, it was common to die from pneumonia, meningitis, infections during childbirth (puerperal fever or "milk fever"), or after surgery.

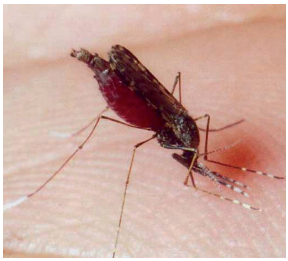
In the tenth century, **Rhazes** made the first clinical description of smallpox (variola virus), and differentiated it from other diseases, such as measles. The interpretation that he gave for the blisters (vesicles) in patients suffering from smallpox was that they had a fermentation of the blood and that the excess

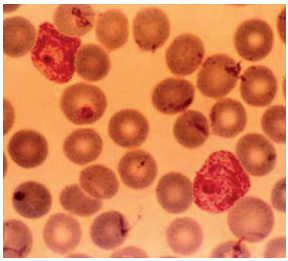
was eliminated by the pox (pustules) (in the same way that grapes transforms into wine).

In **the fifteenth and sixteenth centuries, Girolamo Fracastoro** spoke of contagion through **germs** (little seeds) that pass from one person to another, and would "*germinate*" producing the disease. Given that many diseases, like smallpox and measles, occur in young children, he thought that the children had to have an excess of contaminating maternal blood (from pregnancy and childbirth) that had to be eliminated. The germ would ferment this blood, eliminating it in pustules through the skin.

Nineteenth and twentieth centuries

The great advance in knowledge about infectious diseases took place in the nineteenth century.





Anopheles mosquito that transmits the parasite *Plasmodium Falciparum* malaria producer

Distinguished microbiologists including **Robert Koch** (1843-1910), demostrated that infectious diseases were caused by pathogenic microorganisms, each one of which would cause a specific disease. Koch discovered the causal agents of anthrax, tuberculosis and cholera, and also discovered that insects could transmit some infectious diseases.

Koch outlined some postulates with which all pathogens had to comply to be designated as causal agents of an infectious disease.

KOCH (1843-1910)
Discovered the causal agents of:
- Cholera
- Anthrax
- Tuberculosis

- Discovered that insects transmit trypanosomiasis
- Failed with vaccine tuberculosis

Koch's postulates

1: The microorganism must be present in every case of the disease.

2: The microorganism must be isolated from the host with the disease and grown in pure culture.

3: The specific disease must be reproduced when a pure culture of the microorganism is inoculated into a healthy susceptible host.

4: The microorganism must be recoverable from the experimentally infected host.

Luis Pasteur (1822-1895) was another crucial figure, who disproved and buried the concept of spontaneous generation.

PASTEUR (1843-1910)
- Disproved spontaneous generation
- Fermentation
- Vaccine against cholera
- Vaccine against anthrax
- Vaccine against rabies

Only years later, the causal agents of typhus, diphtheria, tetanus and plague were also discovered

Discovery of causal agents

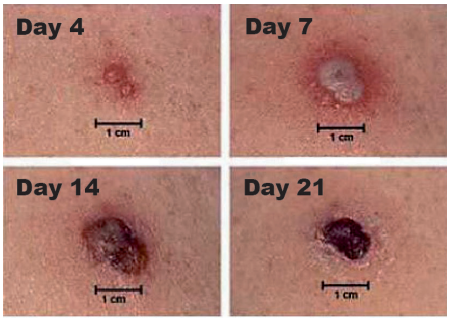
Carbuncle	1876
Typhus	1880
Tuberculosis	1882
Diphtheria	1883
Cholera	1883
Tetanus	1886
Plague	1894

Variolation

Today, smallpox is a disease that has been erradicated throughout the world, but for many centuries it had a high mortality and a worldwide distribution, while those who survived it remained with terrible facial and body lesions.

There was a practice, known as "***variolation***" in China and India (tenth century) that consisted in making children and young people inhale the dry scabs from the pustules of smallpox patients. This practice spread to Constantinople (current Istambul), but there instead of inhaling, small percutaneous punctures were made in the skin (scarification) and pus from the vesicles of the smallpox patients was deposited in them. Although it is not known why this system of applying dry pus was used, some documents of the time indicate it was to "preserve the beauty of our young ladies", without stressing that it saved lives, only that it avoided severe lesions and scars caused by smallpox.

Smallpox evolution



Europe and America were unaware of this practice and suffered a terrible epidemic of smallpox in 1721.

On moving to Constantinople with her husband, the wife of the British ambassador, **Lady Mary Montagu** (her brother had died from smallpox, while the disease left her with severe cutaneous scars, particularly on the eyelids), learnt about variolation and its preventive effects, and convinced her doctor to first inoculate her children and later the British royal family. Initially, the British Court was very reluctant, but after testing it on orphans and prisoners, the variolation was finally approved in what was called the "Royal Experiment" (1722). The success of this variolation in the British royal family helped to spread the practice throughout Europe and America, using the scarification method followed by innoculation of the dry pus from the pustules of pacients.

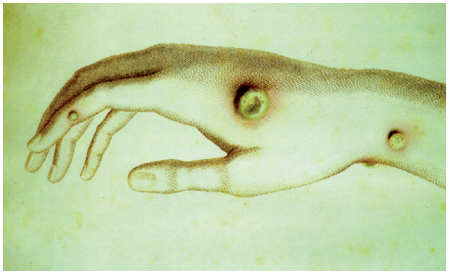
The first vaccine

The beginnings of **Immunology** as a science blossomed in the eighteenth century thanks to an English rural doctor called **Edward Jenner**, who was the first person to produce a vaccine using the scientific method:

Observation: he saw that people milking cows occasionally had pustules on their hands similar to those that sometimes appeared on the udders of cows (cowpox) and they seemed to be like those of human smallpox. Moreover, milk maids and dairy farm workers were not known to suffer smallpox, and in attempting to inoculate them, the smallpox "did not catch" (it did not leave a scar).

Hypothesis. Postulated the idea that **cowpox** could protect from human smallpox.

Experiment. He decided to use the pus from the hand of the milkmaid Sarah Nelmes, who had cowpox lesions, and inoculated a child (James Phipps). Later he tested if it protected from smallpox and innoculated the child with smallpox and he was not taken ill.



The hand and forearm of Sarah Nelmes, as shown in this reproduction of Jenner's book. It states that the large ulcer appeared in May 1796, where there had previously been a scratch. Sara was a young milkmaid.

Dissemination of his results to the scientific community. Although he attempted to publish his work and repeated the experiment with more children, he finally had to pay from his own pocket for the publication of his findings.

Years later and in honour to Jenner, Pasteur would "baptise" the practice of using dead, attenuated or less virulent pathogens to prevent an infectious disease, with the name **vaccination**.

The vaccine against smallpox spread throughout the world, the Royal Philanthropic **Expedition of the Vaccine** promoted by Charles IV (30th November 1803) carried the vaccine from Spain to the Americas. Dr. Balmis was responsible for taking the vaccine to the Spanish colonies and used orphan children from La Coruña to transport the vaccine (innoculating them from arm to arm during the voyage) until they reached the Americas in the Corvete "María Pita".

Vaccination started by Jenner for smallpox was extended to other diseases, such as cholera in chickens, anthrax in cows and rabies in humans, by Louis Pasteur, who based the effect of the vaccination on using pathogens with attenuated virulence. Later, and after learning the causal agents of infectious diseases, vaccines were developed against these agents, during the twentieth century, the golden age of vaccines.

20th - 21th Century Vaccines

	Attenuated	Inactivated	Toxoids / fractions	Polyssacharides	Recombinants
1923			Diphtheria		
1926		Whooping cough			
1927	BCG		Tetanus		
1935	Yellow fever				
1936		Flu			
1950	Smallpox lyophil.				
1955		Polio (Salk)			
1961	Oral Polio (Sabin)				
1963	Measles				
1967	Mumps				
1969	Rubella				
1972				Meningococcal A+C	
1974		Japanese Encephalitis			
1977				Pneumococcal 14-valent	
1978		Polio (improved)			
1979			Hepatitis B (plasma)		
1980	Adenovirus	Rage	European Encephalitis		
1981				Meningococcal 4-valent	
1983	Chickenpox			Pneumococcal 23-valent	
1984				Haemophilus b	
1987				Haemophilus b conjugated	
1988	Cholera				
1989	Thyphoid				Hepatitis B
1990				Cholera	Cholera
1993			Acellular Pertussis		
1994		Hepatitis A			
1995	Chickenpox				
1996					Pertussis
1998					E. Lyme
1999	Rotavirus				
2000				Meningococcal conjugate Pneumococcal 7-valent	
2006					Papillomavirus 6, 11, 16, 18

It was oberved that not only dead or attenuated pathogens could be used to prevent diseases, but also inactivated toxins produced by bacteria, such as those causing tetanus or diphtheria, could be used to provide sufficient protection from the disease. Later, the carbohydrate cover of different bacteria was used to produce vaccines (*Haemophilus, meningococcus* and *pneumococo*), which only give temporary protection. With the arrival of molecular biology and genetic engineering techniques, the first recombinant vaccines could be developed. For these vaccines, DNA sequences are introduced into bacteria, which in turn produce the protein of interest. The first recombinant vaccine was made against the hepatitis B virus.

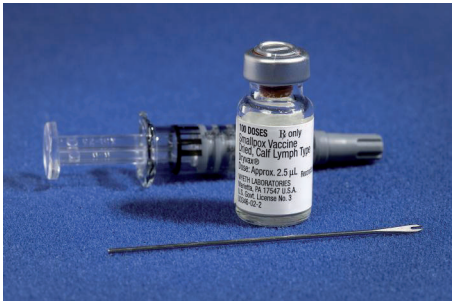
Vaccines that use dead microorganisms
- Bordetella pertussis - Yersinia pestis - Vibrio cholerae - Hepatitis A - Influenza - Poliovirus - Rabies

Vaccines that use attenuated microorganisms
- Mycobacterium tuberculosis - Salmonella typhi - Measles - Poliovirus - Rubella - Mumps - Varicella-zoster

They are produced via passage through animals or in cell cultures

Vaccines that use subunits of microorganisms
- Salmonella typhi - Bordetella pertussis - Clostridium tetani - Corynebacterium diphteriae

Vaccines that use subunits obtained by genetic engineering
- Hepatitis B - Bordetella pertussis - Vibrio cholerae - Borrelia burgdorferi



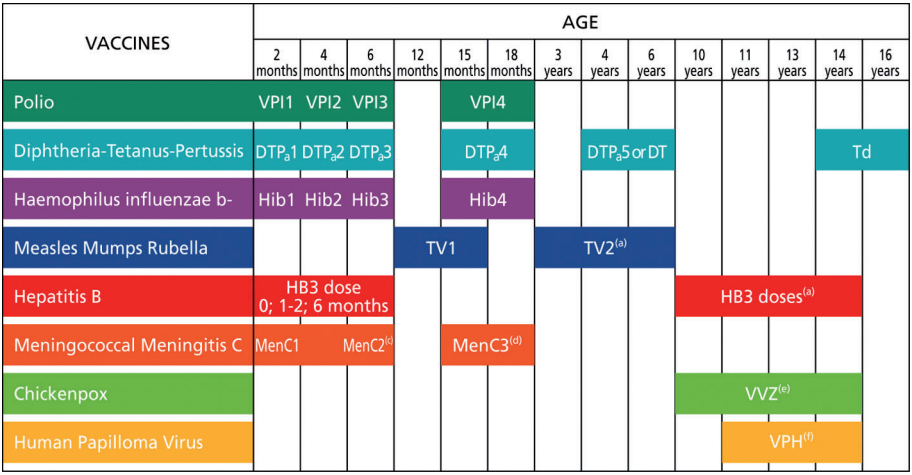
Smallpox vaccine

Vaccines today

Today, vaccines are one of the practices that, together with hygiene, antibiotics and surgery, have saved more lives. In the developed countries, specific vaccination plans are followed for children, with booster doses for some of them at certain times. Other vaccines are administered to the elderly or people in risk groups (such as the annual influenza vaccine or the pneumococcal vaccine). To these conventional vaccines must be added those that have to be administered before travelling to specific countries with a risk of contracting certain diseases.

The vaccination calendar has been changing over the years, and new vaccines have been included. An example of a vaccination calendar is shown in figure.

INTER-TERRITORIAL COUNCIL OF THE NATIONAL HEALTH SYSTEM
RECOMMENDED IMMUNIZATION SCHEDULE (2007) (Spain)
Approved by the Inter-Territorial Council on October 10th, 2007



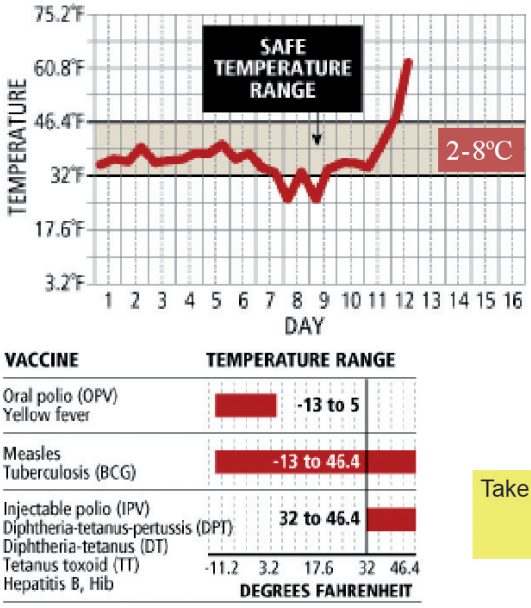
(a) Unvaccinated children in this age range will receive the second dose at 11-13 years.
(b) Children who have not received the primary vaccination in childhood.
(c) Two doses of MenC vaccine between 2 and 6 months apart at least two months.
(d) It is recommended to administer a booster dose after twelve months.
(e) People who report not having had the disease or been vaccinated previously, following instructions on the sheet.
(f) Vaccinate in a single cohort girls between 11-14 years of age.

Routes of administration

Most vaccines are administered by injection (intramuscular or subcutaneous), and only some use the oral or intranasal routes. The use of needles and syringes is a considerable inconvenience, as it is a painful and costly procedure. Therefore, there is an ongoing search for alternative methods of administration of vaccines that do not require injections.

Vaccinating a child in the school of the village, Ethiopia. Bank of images of the Department of Education.
Photo: Pablo María García Lamas

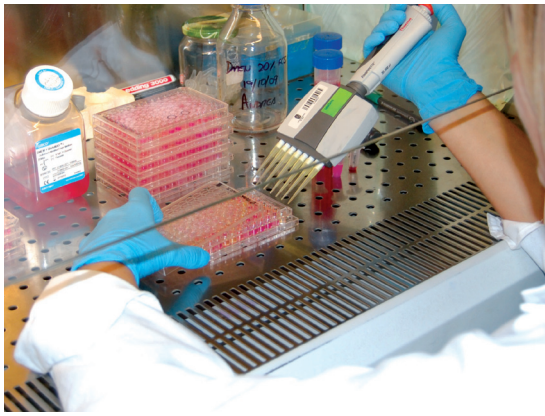
Moreover, as vaccines contain biological components they are very delicate products and most of them need to be kept refrigerated or frozen from when they are produced by the pharmaceutical companies until they reach their destination at health centres, hospitals and dispensaries throughout the world.



Take into consideration:
Storage temperature
Average life

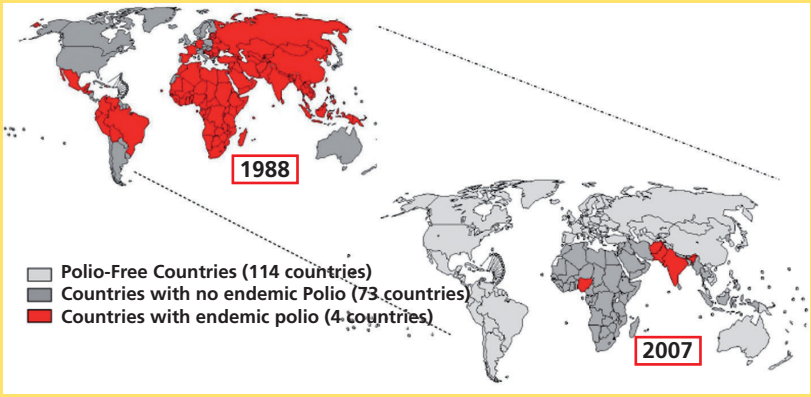
How are vaccines produced?

There are very few companies in the world that comply with the quality criteria necessary for making human vaccines, and with the capacity to be able to produce millions of doses annually. A further difficulty is to actually develop an effective vaccine. The process for producing a conventional vaccine starts with the isolation of the pathogen of interest (for example, the influenza virus), its growth in the laboratory (in chicken eggs, in cell cultures of monkey kidney and lung tissues, or in human diploid cells), its subsequent purification, inactivation or death, followed by toxicity and effectiveness studies, and eventual commercialization. This whole process takes 4-6 months.



Production of vaccines can be a risky high cost practice for the companies involved in their manufacture, as they are biological products and, at times, there are problems with their growth. If there were only one producer in the world, there could be a shortage of vaccines. On other occasions, the pathogen mutates and the vaccine produced is of no use (as occurs each year with the influenza vaccine).

Effect of the vaccine
Progress in Polio Eradication (1988-2007)



What would be the perfect vaccine?

The answer depends on the particular perspective. From the immunological viewpoint, a vaccine must induce memory and an effective and protective response. It is not sufficient that it induces specific antibodies or lymphocytes, it must also demonstrate that it protects from the infection.

However, if patients were asked, the ideal vaccine would be one that did not require injections, or one that could be administered once against a number of infectious diseases, and of course without pain or discomfort.

From the perspective of the Health Authorities of countries, the ideal vaccine would have to be economically viable, as well as easy to produce and distribute worldwide.

Finally, from the social perspective, we wish to eradicate infectious diseases throughout the world, as although one country can eradicate a disease (for example, diphtheria), human migrations between countries mean that its transmission is now very easy. Complete eradication is not an easy task, above all because of the low level of immunization in developing countries. History shows that after so many years of vaccination, only smallpox has been eradicated throughout the world.

The Perfect Vaccine

Immunological
Immune response protective effective/ memory

Patient
No side effects
No need for an injection
Covers numerous pathogens

Economic
Cheap and easily distributed

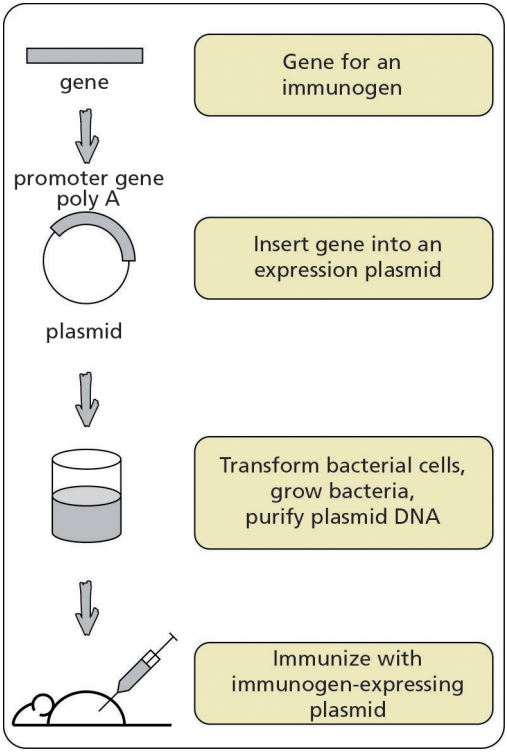
Social
It eradicates a disease throughout the world

New vaccines

In recent years, and thanks to molecular biology and genetic engineering techniques, a new generation of vaccines are being developed that do not require a whole pathogen or even fractions of one, but only need the DNA sequences that encode the proteins of interest of the relevant virus, bacteria or parasite. Moreover, a variety of other new vaccines are being developed including: recombinant vaccines, subunit vaccines, peptide vaccines, DNA vaccines, and those using genetically modified pathogens with low virulence.

DNA vaccine is based on the principle that the individual receiving it produces the proteins of the pathogen. In this method, there is injection of a vector that carries the genes that encode the proteins of the virus or bacterium of interest. This vector is administered in a cutaneous or intramuscular way and the muscular cells or immune cells located under the skin (dendritic cells) can incorporate the DNA and produce the proteins of the virus or bacterium of interest. These proteins of the pathogen produced directly by the individual will activate the immune system, activating the memory and the production of antibodies.

Steps for developing a DNA vaccine



Given that this type of vaccine does not require growing the pathogen, but only knowing its DNA sequences, it is possible to develop it much more rapidly than conventional vaccines, and also to make multivalent ones (for many pathogens at the same time). These vaccines are being tested and compared to the conventional ones, and it is possible that in the future most vaccines will be of this type. In addition, in the event of a pandemic involving a new virus that would require a vaccine quickly, this type of vaccine would definitely be the one obtained most rapidly.

Present and future of vaccines

There are currently several vaccines that function very well in protecting against pathogens, others give less protection or do not function adequately. However, there are viruses, bacteria and parasites for which no effective vaccines have been found. One example is the HIV virus that causes Acquired Immune Deficiency Syndrome (AIDS), which has so far prevented the development of an effective vaccine, because of its characteristics and its very high mutation capability. Therefore, only preventive measures can be advocated for avoiding infection, such as always using condoms in casual sexual relations, avoiding contact with contaminated blood, not sharing needles, sterilizing dental equipment and materials, taking care with tattoos, piercings, etc. There are also no effective vaccines for tuberculosis and malaria, although several clinical trials are being undertaken with promising results and it is expected that, in the near future, effective vaccines will be available for these diseases.

At present, children receive numerous vaccines (most by injection) during the early years of life to protect them from pathogens that could affect them, especially at a young age. The trend for vaccines in the future will see an attempt to simplify the vaccination calendar and reduce the number of injections. Hence, there are already emerging multiple vaccines (against 6-7 pathogens) with this aim, and more pathogens may be incorporated into the same inoculation.

Researchers are also looking for elements that strengthen the immune response to vaccines, which are called ***adjuvants***. Currently, there are very few

Present and future of vaccines

Combined vaccines

Hexavalent vaccine

Diphtheria, Pertussis, Tetanus, Hepatitis B, Polio, Haemophilus

New vaccines

Varicella ("chickenpox"), Rotavirus (gastroenteritis), Herpes, New pneumococcal vaccine

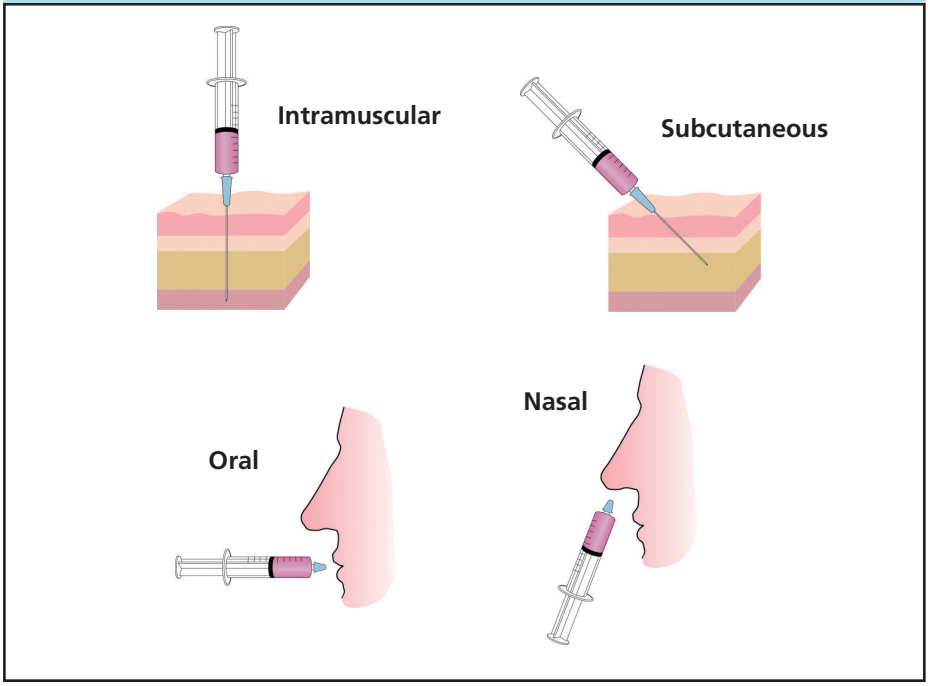
Researching into:

AIDS: High rate of mutation
Tuberculosis: It hides within immunity cells
Malaria: Complex parasite
New routes of administration: Vaccines in aerosol
Genetic vaccines
New adjuvants

authorized for human use. In the United States, only aluminium hydroxide (alum) can be used, which improves recognition by the cells of the immune system that are activated after the vaccination. In Europe, two other adjuvants have been authorized besides alum, but ongoing research is looking for new components (nanostructures, lipids…) that could be used in vaccines.

In the future, vaccines will definitely not be administered by injection, but by oral or intranasal routes. Many researchers are currently studying these possibilities.

Routes of administration



Other types of vaccines (anti-cancer, anti-allergy, etc.)

It is always considered that vaccines protect against a variety of infectious diseases, but they could have other uses. For example, they have already been developed to help cure allergies and tumours and they could be used in the prevention of cancer or to control fertility or smoking. In addition, vaccines are indispensable in the veterinary field for correctly controlling the health of animals, but their potential has also been studied in other areas. One example is the use of vaccines to try to modify the microflora of sheep so that they emit less methane into the atmosphere, thereby helping to protect the ozone layer.

Vaccines can be used to cure allergies. The aim is to modulate the immune system so that it does not respond after the allergen enters or that the allergen does not induce an inflammatory response. Although their use is not indicated in all cases of allergies, vaccines are currently the only effective curative method against allergic processes, because the antihistamines or corticoids that are administered, only modify the symptoms, but do not cure the allergy.

Other types of vaccines

Therapeutic vaccines
Eradicate tumours:
Strengthen the immune response
Vaccines against ALLERGIES

Vaccine-induced prevention of tumours
Prevention of the development of tumours
Genital herpes UTERUS
Hepatitis B (HBV) and C (HCV) LIVER
Human papillomavirus (HPV) GENITALS
Heliobacter pylori (HP) STOMACH

Other uses
To control fertility
To stop smoking (vaccine against nicotine)

In the field of tumours, it is more and more clear that certain viruses have a role in the development of cancer. Relationships have been demonstrated between genital herpes and uterine cancer, between the bacteria Helicobacter pilory and stomach cancer, Hepatitis B virus and liver cancer, and more recently between human papillomavirus and uterine cancer and that of other genital organs. Therefore, immunization against these pathogens not only would protect from infection, but also would protect against later development of cancer.

In the specific case of papilloma, more than 100 different types of human papillomavirus are known, but only some of these (6, 11, 16 and 18) cause cervical cancer. The recently approved vaccine should be administered before infection and, therefore, before the person contracts the virus. Given that this virus is sexually transmitted, it is advisable that the vaccine is used for girls of 13-14 years of age, before they become sexually active.