**VACCINES**

**IMMUNIZATION**

A person can become immune to a certain infectious disease with the help of two artificial methods:

**Active immunization** is the administration (inoculation) of antigens that induce a specific immune response.

**Passive immunization**, by which the patient acquires temporary resistance to a specific infection, involves the transfer of preformed antibodies from another individual (human or animal).

Active immunization directed at a specific microorganism is called **vaccination**. It was named in honor of Edward Janner's pioneering discovery in 1796 that smallpox can be prevented by inoculating the cowpox virus (*Vaccinia*, Latin *Vacca* - cow). Although Louis Pasteur showed in 1980 that immunization is also possible against other infectious diseases, the term vaccination has persisted to this day. The development of a specific immune response after vaccination is based on the property of cells of acquired immunity (B and T lymphocytes) that differentiate into memory cells after contact with the antigen. The function of the vaccine is to induce a memory immune response without causing disease, so that the microorganism after the first contact with the patient induces a more effective, secondary, rather than primary immune response. Vaccines can act on the activation and differentiation of either T or V lymphocytes, although most induce a response of both types of lymphocytes. The immune response induced by vaccines includes:

• production of antibodies (IgG and IgM) for extracellular bacteria (opsonization, phagocytosis, complement), bacterial exotoxins (neutralization) and viruses

• production of mucosal antibodies (IgA) for extracellular bacteria (adherence inhibition) and viruses

• induction of cellular immunity (T lymphocyte - macrophage) for intracellular bacteria and viruses

**CHARACTERISTICS OF VACCINE**

There are no vaccines against all diseases, and the reason for this is that, in order to be used, a vaccine must meet the following criteria. The vaccine must be:

**Efficient**

The ideal vaccine is highly immunogenic and provides complete and long-term (lifelong) protection against infection. Thanks to successful vaccination, some diseases such as smallpox and diphtheria have been declared eradicated today. Other diseases, such as smallpox, rubella, mumps, poliomyelitis or tetanus, are expected to be eradicated in the near future. However, despite existing vaccines, numerous infectious diseases remain a significant medical problem. Examples include influenza, rabies and tuberculosis. The reasons for the reduced effectiveness of vaccines against these diseases are different. For example, the influenza virus is subject to intense antigenic variations, so it is very difficult to match the administered vaccine with the current strain of the disease-causing agent. Finally, for a large number of infectious diseases today, there are still no effective vaccines (eg, the common cold, staphylococcal infections, as well as virtually all fungal and parasitic infections) (Table 1).

**Table 1.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **viruses** | **bacterias** | **fungi** | **parasytes** |
| **Vaccines in general use** | Poliomyelitis | Difteria  Tetanus  Pertussis |  |  |
|  | Morbili  Mumps  Rubella | BCG  (in some countries) |  |  |
|  | Hepatitis B |  |  |  |
| **Vaccines that are given to people who are at increased risk of getting the disease** | Influenza | BCG |  |  |
|  | Yellow fever | Typhus |  |  |
|  | Hepatitis A | Pneumococcus |  |  |
|  | Rabies | Meningococcus |  |  |
|  | Varicella-zoster virus | Haemophilus |  |  |
|  |  | Antrax |  |  |
| **Vaccines not yet available for use** | Adenovirus | Staphylococcus | Candida | Malaria |
|  | Rhinovirus | Streptococcus | Pneumocystis | Leishmania |
|  | Herpes virusi | Gonococcus |  | Schistosomiasis |
|  | Respiratory syncicial virus (RSV) | Syphilis |  | Filaria |
|  | HIV | Leprosy |  |  |

**Safe**

Administering vaccines is the only medical treatment administered to healthy people. Hence, any accident related to vaccination represents a very important and expensive health problem. The main side effects and limitations to the use of vaccines are shown in Table 2.

**Table 2.**

|  |
| --- |
| **Limitations and problems with vaccines** |
| Inactivated vaccines - microorganisms are not adequately killed |
| Attenuated microorganisms can transform into wild type and regain virulence (eg polioviruses types 2,3) |
| Inclusion of toxic material (eg typhoid, whooping cough) |
| Contamination with animal viruses |
| Egg protein contamination (hypersensitivity, allergy) |
| Cross reaction with one's own (autoimmune diseases) |
| **Limitations and problems on the part of the patient** |
| Immunodeficiency (live microorganisms can cause serious diseases) |
| Local inflammatory reaction, often to an adjuvant |
| Vaccine hypersensitivity (eg tetanus) |
| Interference between vaccines given at the same time (not always) |

The most common problem associated with vaccines is their mild toxicity. Some vaccines, especially adjuvanted whole vaccines, may cause pain at the site of inoculation for several hours or days after administration. In rare cases, toxicity may lead to a systemic reaction followed by malaise and fever. A much more serious problem is the possible occurrence of an anaphylactic reaction to one of the components of the vaccine, such as egg proteins (eg, the flu vaccine where the causative agents are grown in chicken embryos), adjuvants or condoms. A third significant problem associated with immunization is the residual virulence of the pathogen. Attenuated viruses can occasionally cause disease not only in immunodeficient individuals or fetuses, but also in healthy children and adults. A good example of this is the live attenuated polio vaccine. Although it is a very effective vaccine, one in 2 million children develop clinically manifest disease after vaccination. This problem was overcome by introducing an inactivated, dead vaccine into use.

**Stable**

The stability of vaccines is particularly important, given that vaccines are most often administered in environments far from the place of their production. This problem is particularly significant when it comes to live vaccines.

**Cheap**

Most vaccines are very cheap, so vaccination as a form of prevention of infectious diseases is considered the most cost-effective. However, in many parts of the world, vaccine administration is still a significant economic problem. There are also vaccines that are still very expensive to produce (eg hepatitis V vaccine).

Scientists are constantly striving to create the most effective and safe vaccine. In either case, the pathogen is altered or inactivated so that it cannot cause disease. However, not all vaccines are equally effective and safe. Efficacy can be monitored by determining the titer of antibodies (IgG and IgM) in the blood. If the antibody level is low, it can be restimulated by giving a larger amount of antigen - booster immunization.

**TYPES OF VACCINES**

Vaccines, each of which has its own advantages and disadvantages, are divided according to the type of antigen they contain. Vaccines can generally be divided into three groups:

1. **Live vaccines** containing live microorganisms can be: attenuated, virulent and recombinant.
2. **Dead vaccines** can be inactivated (contain whole microorganisms) or subunit (contain antigenic fragments), which are divided according to the origin of the antigen into purified, synthetic or recombinant.
3. **DNA vaccines** that do not contain microorganism antigens but contain DNA sequences that encode protective microorganism antigens.

Examples are shown in Table 3.

**Table 3.**

|  |  |
| --- | --- |
| Type of vaccine | **Examples** |
| **Live vaccines** | Smallpox, measles, mumps, rubella, poliomyelitis (Seibin vaccine), yellow fever, varicella, BCG, cholera, rotavirus |
| **Dead (inactivated) vaccines containing whole pathogens** | Rabies, influenza, poliomyelitis (Salk vaccine) hepatitis A, pertussis, typhoid, cholera |
| **Dead (subunit) vaccines containing antigenic fragments (protein, polysaccharide, conjugated)** | Meningococcus, Pneumococcus, H. influenzae, hepatitis V, tetanus, diphtheria, pertussis |
| **DNA vaccines** | \*Influenza, HIV |

**Live attenuated vaccines** contain attenuated microorganisms and are also called modified live vaccines. They contain active (live) but avirulent microorganisms, so under normal conditions they can cause mild infections, but not clinically developed disease. Attenuation of microorganisms is the process of induction of spontaneous mutations of the pathogen's genome after exposure to unfavorable environmental conditions (eg, low temperature, lack of nutrients, etc.) and the selection of mutant strains that have lost virulence, but preserved antigenicity (Figure 1).

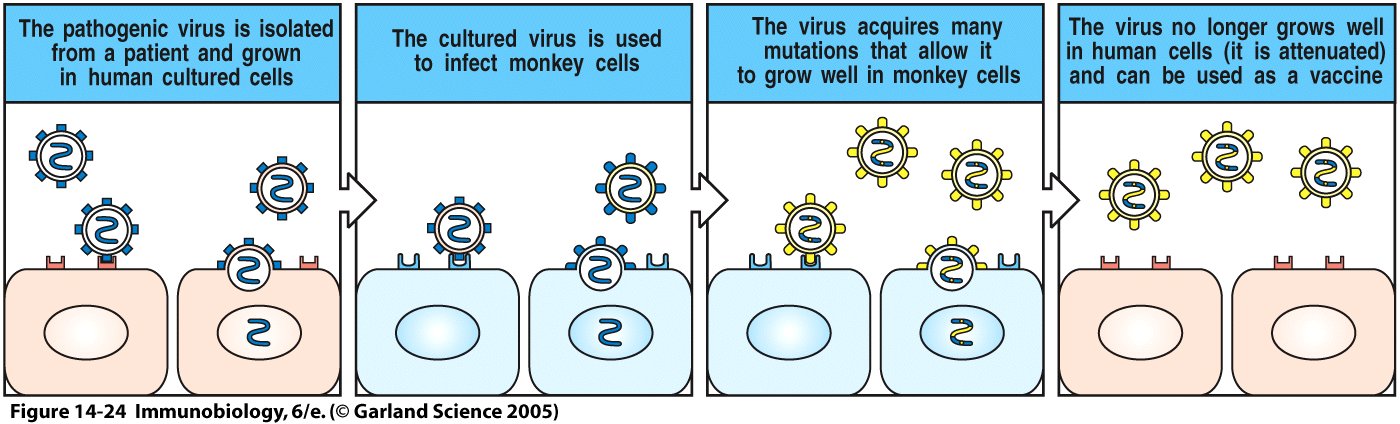


Figure 1. To obtain an attenuated virus, it is first grown in human cell culture. After that, the same strain is adapted to grow in cell culture of another species until it loses its ability to grow in human cells. Adaptation is the result of point mutations of the viral genome. The attenuated virus grows slowly in human cell culture - inducing an immune response but not disease.

**Live virulent vaccines** contain virulent microorganisms that are not pathogenic for humans, but the similar antigenic composition of related viruses (animal and human) enables the induction of a cross-immune response. A classic example of a live virulent vaccine is the smallpox virus (*v. variolae*) vaccine, which contains the genetically related cowpox virus (*v. vacciniae*).

**Live recombinant vaccines** contain live pathogens that have been modified by recombinant techniques so that they cannot cause clinically developed disease, but can trigger an immune response. This group includes vaccines. Vector vaccines containing live avirulent viruses (vectors) into which the gene of the virulent virus responsible for the synthesis of the protective antigen has been inserted. In the vaccinated organism, the vector multiplies and synthesizes a sufficient amount of antigen. The most commonly used vectors are v. vacciniae and other poxviruses which, due to their large genome, are suitable for the insertion of a new gene (eg adenovirus, herpesvirus, poxvirus and salmonella vaccines).

- Deletion mutants containing live infectious viruses in which genes encoding virulence factors have been mutated or removed by recombinant techniques, resulting in an irreversibly attenuated microorganism (Figure 2).

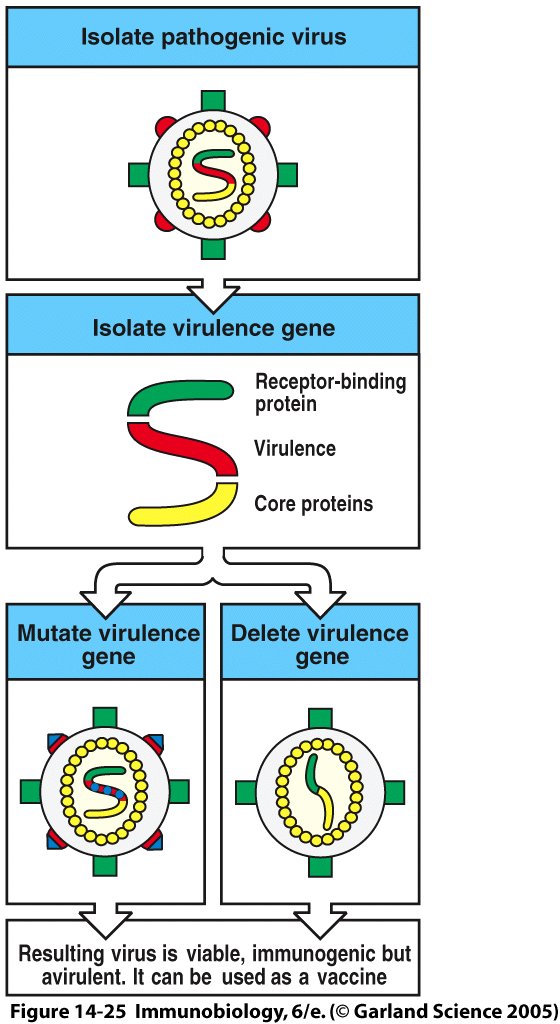


Figure 2. If a viral gene responsible for virulence, but not growth and immunogenicity, is identified, it can be mutated or removed by recombinant DNA techniques. This virus is avirulent and can be used as a vaccine

Live vaccines are most often given to mimic the natural route of infection. Given that live vaccines contain active microorganisms, a large number of antigens are able to induce an effective immune response, so these vaccines activate both humoral and cellular immune responses, stimulate interferon production, and provide good and long-lasting protection. In addition to numerous advantages, live vaccines also have disadvantages. They are less stable than dead vaccines and require special storage conditions. Vaccinated persons can be infectious to others in the environment, the so-called. contact immunity. Although very effective, attenuated vaccines can be very dangerous for immunocompromised patients, ie. modified microorganisms can retain residual virulence and cause disease in immunocompromised individuals. Live vaccines should not be given to pregnant women due to the possibility of pathogens crossing the placental barrier and causing fetal infection. Sometimes, modified viruses can regain virulence or mutate into a form that can cause disease, so a strict risk assessment is recommended for the use of these vaccines.

**Dead vaccines** are divided into two subtypes: **inactivated vaccines**, which contain inactivated whole microorganisms, and **subunit vaccines**, which consist of antigenic fragments of microorganisms. These vaccines are safer than live vaccines because dead pathogens or their antigens cannot replicate, have no residual virulence, and cannot mutate into a wild strain. However, since inactivated microorganisms cannot replicate, several booster doses are often required to develop a complete immune response. Administration of dead vaccines cannot cause contact immunity. Non-antigenic parts of microorganisms can sometimes contribute to painful inflammation. Therefore, today the use of dead pertussis causative agent is replaced by subunit vaccine.

Such vaccines are antigenically weak stimulators of the immune response, so they must be given in larger or repeated doses, which can lead to the appearance of allergic reactions. A special approach to this problem is the addition of chemical compounds called **adjuvants**, which increase the immunogenicity of antigens and are used as additives to make inactivated vaccines. Adjuvants increase the effectiveness (antigenicity) of the vaccine by stimulating and activating Toll-like receptors. By themselves, some cytokines such as IL-1, IL-2 and IFN-γ may have adjuvant activity. Artificial lipid vesicles known as liposomes are used to transport antigens to antigen-presenting cells. An adverse reaction that occurs as a result of the increase in antigenicity of the vaccine is the possibility of local inflammation.

**Inactivated vaccines.** When inactivating pathogens, it is important that antigens for immunization remain as similar as possible to those of living microorganisms. Most often, heat or formaldehyde is used for inactivation to denature proteins and nucleic acids. Given that inactivated microorganisms in dead vaccines cannot reproduce, they present a smaller number of antigens than live vaccines. Given that the immune system recognizes dead pathogens of inactivated vaccines as exogenous antigens, they primarily stimulate the development of humoral immunity and the production of antibodies.

**Subunit vaccines**. According to the origin of the antigens they contain, these vaccines are divided into purified, synthetic or recombinant. Purified vaccines are obtained from cultures of microorganisms and contain purified and concentrated antigens of the microorganisms. Synthetic vaccines contain synthetic peptides whose amino acid sequence is identical to the peptides of microorganisms. Recombinant vaccines contain purified antigens obtained by recombinant techniques. These techniques yield large quantities of highly pure viral or bacterial antigens. In this process, an isolated target gene is inserted into the genome of a bacterium, yeast, or other cell. The cloned vector in vitro produces large amounts of antigen, which is then purified. In this way, the hepatitis V vaccine is produced in yeast cells.

**Toxoids as vaccines.** For some bacterial infections, it is more effective to induce an immune response by administration of toxins than by cellular antigens (eg, tetanus and diphtheria). Toxoids are chemically and temperature modified bacterial toxins that have retained their antigenicity. As with dead vaccines, toxoids induce a humoral immune response and the production of specific antibodies. Since toxoids have fewer antigenic determinants, effective immunization requires repeated doses of vaccination in childhood, as well as revaccination every 10 years throughout life.

**Combined vaccines** consist of a combination of several toxoids and inactivated pathogens administered simultaneously. Several combination vaccines are in general use. Examples are the MMR vaccine, which contains attenuated measles, mumps, and rubella, or the DiTePer vaccine, which contains diphtheria and tetanus toxoids, as well as dead pertussis.

**DNA vaccines**. Although the vaccines used so far have proven to be effective, scientists are constantly searching for a more effective, safer and cheaper vaccine, as well as the creation of vaccines that are not yet available in medical practice. The latest method of immunization involves the inoculation of a DNA sequence that encodes an antigen, instead of the administration of the antigen itself (eg hemagglutinin of the influenza virus). The DNA encoding the antigen of the pathogen is inserted into a plasmid, which is inoculated into the patient's body as a vector. The cells of the organism take up the plasmid and transcribe it. The product of the gene encoding the antigen induces a cellular immune response (Figure 3). In this way, the vaccinated organism develops an immune response to the heterologous protein produced by its own cells.

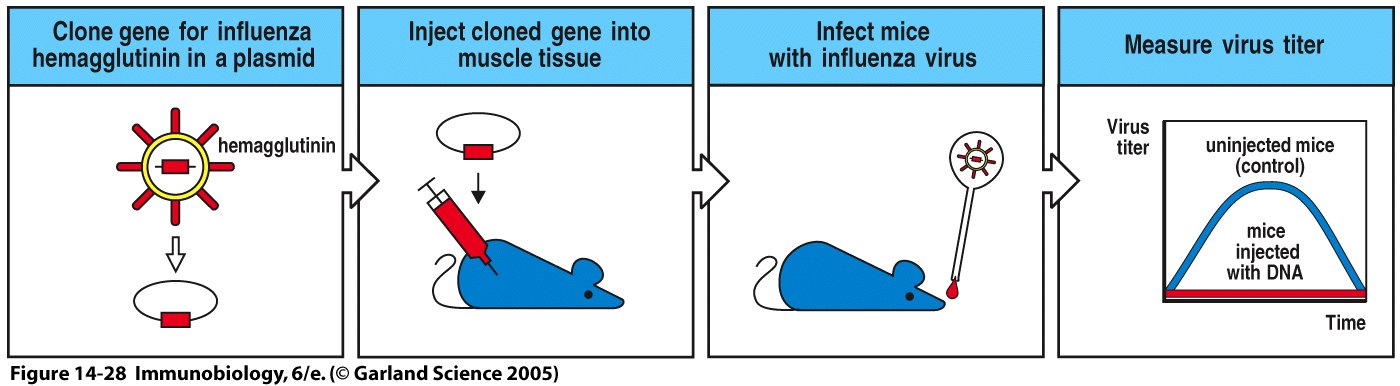


Figure 3.

The advantages of DNA vaccines are numerous. Plasmids can be obtained easily and in large quantities. Plasmid production is cheap and plasmid DNA is stable, making it easy to transport and store. Plasmid DNA sequences can be easily changed, which allows a rapid response to changes in the infectious agent (seasonal variations of the influenza virus). Proteins synthesized on the basis of the plasmid DNA sequence are post-translationally modified in our cells in the same way as during natural infection. Such an antigen is superior to antigens produced in expression vectors under in vitro conditions. In addition, mixtures of plasmids can be used, which allows the preparation of combined vaccines. DNA vaccines potentially have fewer side effects. The plasmid does not replicate and only encodes the protein of interest, so no immune response to unwanted antigens or the vector microorganism develops. The antigen obtained in this way is presented as part of the first-class GHK molecule, which enables the activation of the cellular immune response and the generation of cytotoxic lymphocytes. The potential disadvantages of these vaccines are related to the possibility of integration of the plasmid into the genome of the host cells, which may result in the occurrence of insertional mutagenesis, the induction of an autoimmune response or even the induction of immune tolerance to the antigen.