

Immunization is a means of providing specific protection against pathogens by stimulating an organism's immune system to either produce humoral antibodies against the pathogen or T cells that can provide cell-mediated immunity.

The type of immunity that is needed to neutralize a specific pathogen depends on the site of the pathogen and the mechanism of its pathogenesis. For example, some pathogens produce disease by secreting exotoxins. If this is the case, the only immune mechanism effective against the organism would be **neutralizing antibodies that prevent exotoxin binding to the appropriate receptor on its target cell** and promoting its clearance and degradation by phagocytes.

If the pathogen produces disease by other means, an antibody will have to react with the pathogen itself and eliminate it either by complement-mediated lysis or phagocytosis and intracellular killing.

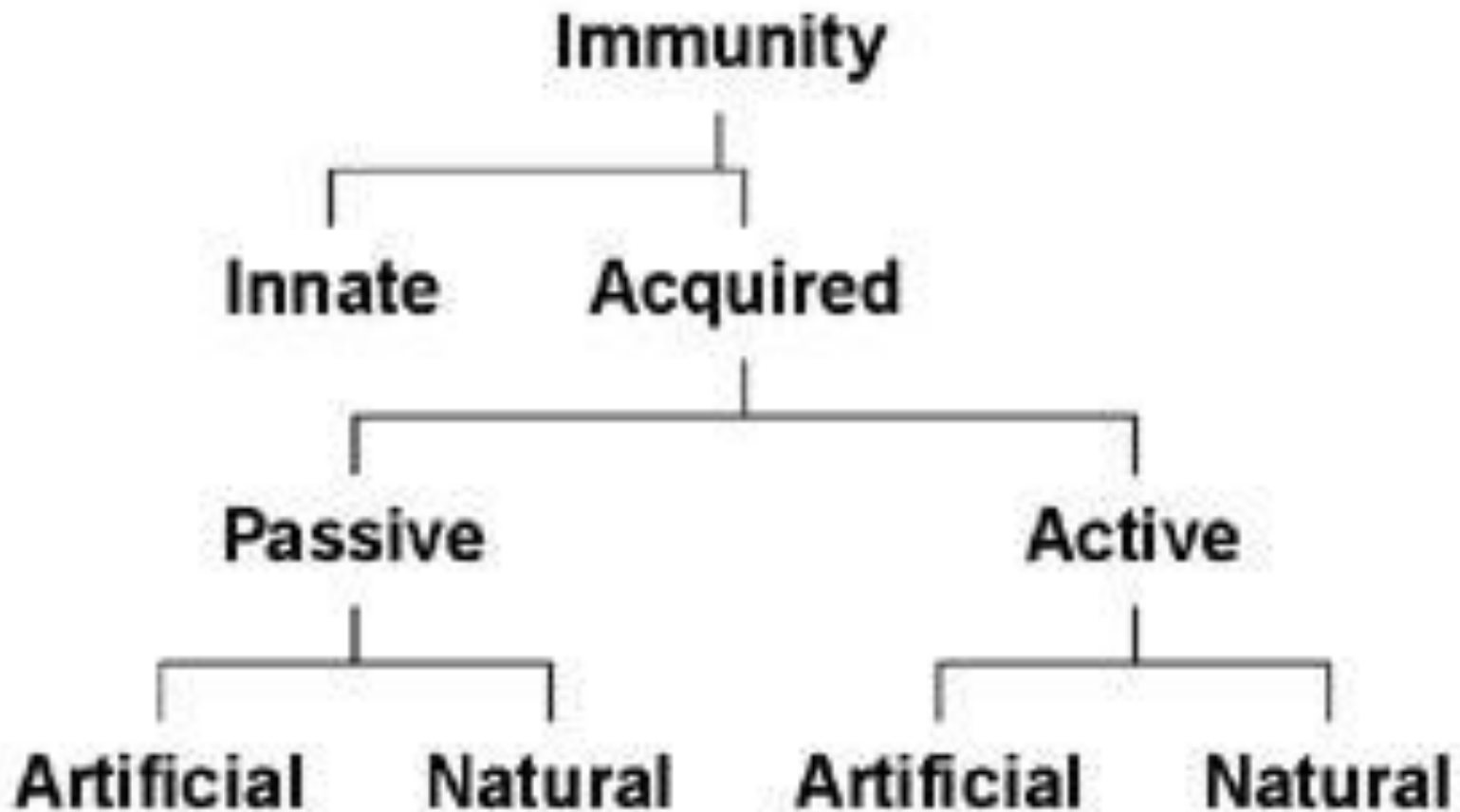
However, if the pathogenic organism is localized intracellularly, it will not be accessible to antibodies and the cell harboring it will have to be destroyed instead. Most viruses, together with intracellular bacteria and protozoa, are examples of such pathogens. In this case, the harboring cells can be destroyed by elements of cell-mediated immunity or, if they cause the infected cell to express unique antigens recognizable by antibody, antibody-dependent and complement-mediated killing of the infected cell can expose the pathogen to elements of humoral immunity.

Edward Jenner carries out a vaccination





Modes of immunization



Specific immunity can result from either passive or active immunization and both modes of immunization can occur by natural or artificial processes

PASSIVE IMMUNITY

Immunity can be acquired, without the immune system being challenged with an antigen. This is done by transfer of serum or gamma-globulins from an immune donor to a non-immune individual. Alternatively, immune cells from an immunized individual may be used to transfer immunity. Passive immunity may be acquired naturally or artificially.

Naturally acquired passive immunity

Immunity is transferred from mother to fetus through placental transfer of IgG or [colostral](#) transfer of IgA.

Artificially acquired passive immunity

Immunity is often artificially transferred by injection with gamma-globulins from other individuals or gamma-globulin from an immune animal. Passive transfer of immunity is used in numerous acute situations of infection (diphtheria, tetanus, measles, rabies, etc.), poisoning (insects, reptiles, botulism), and as a prophylactic measure ([hypogammaglobulinemia](#)). In these situations, gamma-globulins of human origin are preferable, although specific antibodies raised in other species are effective and used in some cases (poisoning, diphtheria, tetanus, gas gangrene, botulism). While this form of immunization has the advantage of providing immediate protection, heterologous gamma-globulins are effective for only a short duration and often result in pathological complications ([serum sickness](#)) and [anaphylaxis](#). Homologous immunoglobulins also carry the risk of transmitting hepatitis and HIV.

Passive transfer of cell-mediated immunity can also be accomplished in certain diseases (cancer, immunodeficiency). However, it is difficult to find histocompatible (matched) donors and there is severe risk of graft versus host disease.

ACTIVE IMMUNITY

This refers to immunity produced by the body following exposure to antigens.

Naturally acquired active immunity

Exposure to various pathogens leads to sub-clinical or clinical infections which result in a protective immune response against these pathogens.

Artificially acquired active immunity

Immunization may be achieved by administering **live or dead pathogens** or their components. Vaccines used for active immunization consist of live (attenuated) organisms, killed whole organisms, microbial components or secreted toxins (which have been detoxified).

Milestones in immunization

◆ 3000BC

- ◆ Evidence of sniffing powdered small pox crust in Egypt

◆ 2000BC

- ◆ Sniffing of small pox crust in China

◆ 1500BC

- ◆ Turks introduce variolation

◆ 1700AD

- ◆ Introduction of variolation in England and later in the US

◆ 1780AD

- ◆ Edward Jenner discovers small pox vaccine

◆ 1885AD

- ◆ Pasteur discovers rabies attenuated vaccine



Рис. 4. Первая в мире вакцинация против бешенства, выполняемая ассистентом Пастера

Introduction of variolation

The wife of the British Ambassador in Turkey, in March 1717 wrote, following the variolation of her son, to a friend in England: “The small pox, so fatal, so general amongst us, is entirely harmless here by the invention of ingrafting....I am patriot enough to bring this invention into fashion in England.

Live vaccines

The first live vaccine was **cowpox virus** introduced by Edward Jenner as a vaccine for smallpox; however, **variolation** (innoculation using pus from a patient with a mild case of smallpox) has been in use for over a thousand years.

Live Attenuated Vaccines

Live vaccines are used against a number of viral infections (polio (Sabin vaccine), measles, mumps, rubella, chicken pox, hepatitis A, yellow fever, etc.)

- polio*
- not used in std. schedule
- measles, mumps & rubella
- Varicella zoster
- children with no history of chicken pox
- tuberculosis
- not used in this country
- hepatitis A
- not required in SC
- yellow fever
- Military and travelers

The example of live bacterial vaccine is one against tuberculosis (*Mycobacterium bovis*: **Bacille Calmette-Guerin vaccine: BCG**). This is used in many African, European and Asian countries. Whereas many studies have shown the efficacy of BCG vaccine, a number of studies also cast doubt on its benefits.

Live vaccines normally produce self-limiting non-clinical infections and lead to subsequent immunity, both humoral and cell-mediated, the latter being essential for intracellular pathogens. However, they carry a serious risk of causing overt disease in immunocompromised individuals. **Furthermore, since live vaccines are often attenuated by passage in animals or thermal mutation, they can revert to their pathogenic form and cause serious illness.** It is for this reason that live polio (Sabin) vaccine, which was used for many years, has been replaced in many countries by the inactivated (Salk) vaccine.

Killed Whole-Organism Vaccines

— polio

— Q fever

— population at risk

— influenza

— typhoid, cholera, plague

— elderly and at risk

— epidemics and travelers

— rabies

— pertussis

— post exposure

— replaced by the
acellular vaccine

Killed vaccines

Killed (heat, chemical or UV irradiation) viral vaccines include those for polio (Salk vaccine), influenza, rabies, etc. Most bacterial vaccines are killed organisms (typhoid, cholera, plague, pertussis, *etc.*)

Microbial Fragment Vaccines

— *Bordetella. Pertussis*

— virulence factor protein

— *Haemophilus influenzae B*

— protein conjugated polysaccharide

— *Streptococcus pneumoniae*

— Polysaccharide mixture

— *Neisseria meningitidis*

— polysaccharide

Sub-unit vaccines

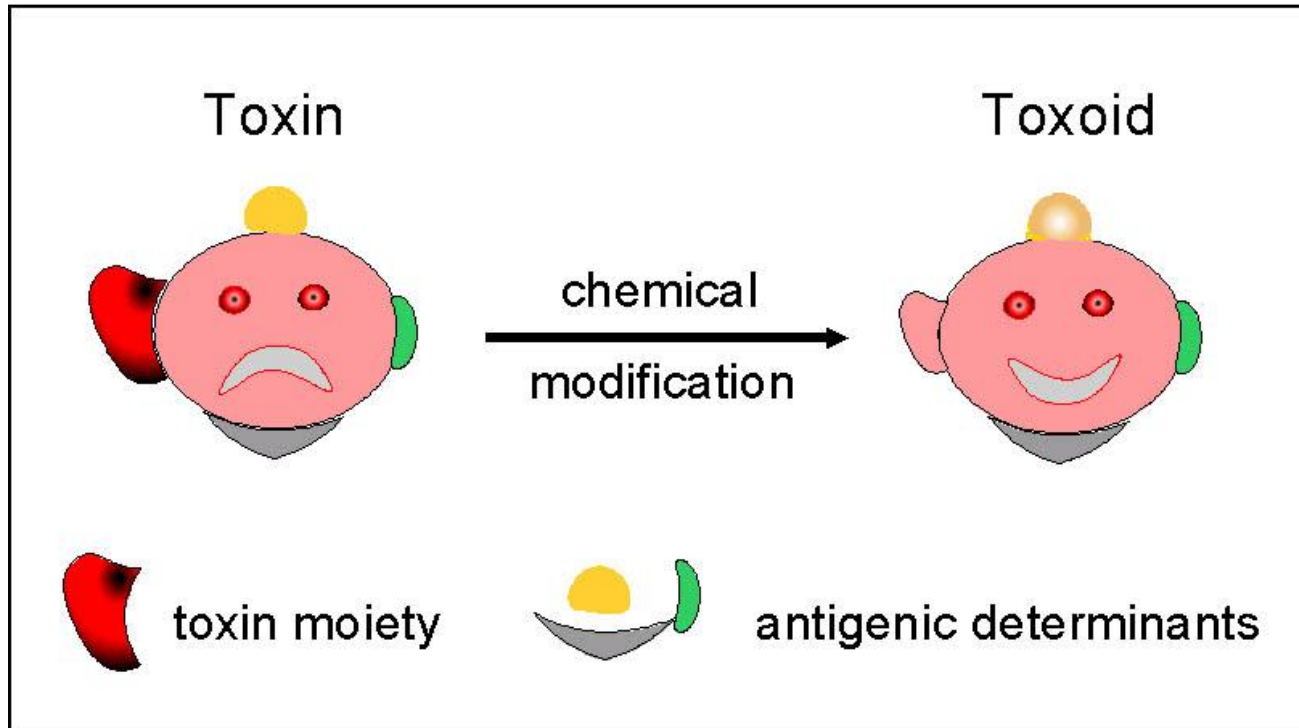
Some anti-bacterial vaccines utilize purified cell wall components (haemophilus, pertussis, meningococcus, pneumococcus, etc.) Some viral vaccines (hepatitis-B, etc.) consist of purified antigenic proteins manufactured after expression from a gene cloned into a suitable vector (e.g., yeast).

Microbial Fragment Vaccines

- ☛ *Clostridium tetani* (tetanus)
 - ☛ inactivated toxin (toxoid)
- ☛ *Corynebacterium diphtheriae*
 - ☛ inactivated toxin (toxoid)
- ☛ *Vibrio cholerae*
 - ☛ toxin subunits
- ☛ Hepatitis B virus
 - ☛ cloned in yeast

Subunit vaccines may consist of proteins or polysaccharides. Since polysaccharides are relatively weak T-independent antigens, and produce only IgM responses without immunologic memory, they are made more immunogenic and T-dependent by conjugation with proteins (e.g., haemophilus, meningococcus, pneumococcus, etc.).

Modification of Toxin to Toxoid



When the pathogenic mechanism of an agent involves a toxin, a modified form of the **toxin (toxoid, which has lost its toxicity while remaining immunogenic)** is used as a vaccine (*e.g.*, diphtheria, tetanus, cholera).

These subunit vaccines are designed to reduce the toxicity problems. Each type of vaccine has its own advantages and disadvantages.

1796	НАТУРАЛЬНАЯ ОСПА
1885	БЕШЕНСТВО
1896	ХОЛЕРА, ЧУМА
1921	ТУБЕРКУЛЕЗ
1923	ДИФТЕРИЯ
1926	КОКЛЮШ
1927	СТОЛЬНЯК
1935	ЖЕЛТАЯ ЛИХОРАДКА
1936	ГРИПП
1939	КЛЕЩЕВОЙ ЭНЦЕФАЛИТ
1941	СЫПНОЙ ТИФ
1951	БРУЦЕЛЛЕЗ


1954	ПОЛИОМИЕЛИТ
1963	КОРЬ
1967	ПАРОТИТ
1969	КРАСНУХА
1972	МЕНИНГИТ
1976	ПНЕВМОКОКК
1984	ВЕТРЯНАЯ ОСПА
1986	ГЕПАТИТ В
1991	ГЕПАТИТ А
1998	РОТАВИРУСНАЯ ИНФЕКЦИЯ
1998	БОРЕЛИОЗ

Advantages and Disadvantages of Passive Immunization

Advantages

-  immediate protection

Disadvantages

-  no long term protection
-  serum sickness
-  risk of hepatitis and Aids
-  graft vs. host disease (cell graft only)

Adverse event occurring within 48 hours DTP vaccination

Event	Frequency
Local: redness, swelling, pain	1 in 2-3 doses
Mild/moderate systemic: fever, drowsiness, fretfulness vomiting, anorexia	1 in 2-3 doses 1 in 5-15 doses
More serious systemic: persistent crying, fever collapse, convulsions acute encephalopathy permanent neurological deficit	1 in 100-300 doses 1 in 1750 doses 1 in 100,000 doses 1 in 300,000 doses

Prophylactic versus therapeutic immunization

Most vaccines are given prophylactically, i.e. prior to exposure to the pathogen. However, some vaccines can be administered therapeutically, i.e. post exposure (e.g., rabies virus). The effectiveness of this mode of immunization depends on the rate of replication of the pathogen, incubation period and the pathogenic mechanism.

In a situation where the pathogen has a **short incubation period**, only a small amount of pathogenic molecules could be fatal (e.g., tetanus and diphtheria); **therefore both passive and active post exposure immunization are essential**. This is also the case when a bolus of infection is relatively large.

Passive prophylactic immunization is also normal in cases of defects in the immune system, such as hypogammaglobulinemias.

Adverse effects of immunization

Active immunization may cause fever, malaise and discomfort. Some vaccine may also cause joint pains or arthritis ([rubella](#)), convulsions, that may sometimes be fatal ([pertussis](#)), or neurological disorders ([influenza](#)).

Allergies to eggs may develop as a consequence of viral vaccines produced in eggs (measles, mumps, influenza, yellow fever). Booster shots result in more pronounced inflammatory effects than the primary immunization. The serious side effects have been documented after use of the **DTP vaccine** (next Table). Most of these were attributable to the whole pertussis component of the vaccine and have been eliminated by the use of an **acellular pertussis preparation**.

**Table 3. Approximate rates of adverse event occurring within 48 hours
DTP vaccination**

Event	Frequency
Local	
redness, swelling, pain	1 in 2-3 doses
Mild/moderate systemic	
fever, drowsiness, fretfulness	1 in 2-3 doses
vomiting, anorexia	1 in 5-15 doses
More serious systemic	
persistent crying, fever	1 in 100-300 doses
collapse, convulsions	1 in 1750 doses
acute encephalopathy	1 in 100,000 doses
permanent neurological deficit	1 in 300,000 doses

BIOTECHNOLOGY OF VACCINES

Vaccines. Vaccines are substances, derived from a pathogen, that are used to stimulate an animal's immune system to produce the antibodies needed to prevent infection from that particular pathogen. Vaccination is therefore the main approach to protect animals from infectious diseases. The majority of vaccines are based on material directly derived from inactivated bacteria or viruses, which potentially revert to their virulent (disease-causing) form.

Modern biotechnology offers possibilities to engineer specific vaccines that are free from pathogen-derived material. One approach is based on **recombinant protein technology**: once a protein from a pathogen that serves as antigen has been identified, this protein can be safely expressed in cell culture, e.g. in *E. coli* or mammalian cells, using recombinant DNA technology. Subsequently, this protein can be harvested, purified and used as a vaccine. In addition, it has also become possible to create fusions of several pathogen proteins, so that one final protein stimulates a variety of immune responses.

A second approach consists of using **DNA-based vaccines**. This methodology is based on the delivery of plasmid DNA to the cells of a host animal that encodes pathogenic proteins. Once expressed within the cell, the proteins stimulate the animal's immune response in the same way as if the proteins were delivered from outside; thus the animal serves as its own bioreactor for vaccine production. The efficiency of this method is largely dependent on effective plasmid delivery to the animal cells; methods for delivery include *chemical transformation, electroporation, injection and the gene gun*.

BIOTECHNOLOGY OF VACCINES

A third approach is the delivery of pathogen-derived antigens by **live recombinant vectors**.

Bacteria, viruses or even parasites can be engineered to express foreign proteins from the pathogen of interest that act as antigens. The engineered organism is then delivered to the animal, where it induces a limited infection and presents the foreign pathogenic protein, thus stimulating an immune response against that pathogen.

Recently, a very interesting **combination of transgenic plant technology and animal vaccination has emerged**: plants are engineered to express an antigenic protein from a pathogen at high levels in their tissues or storage organs. Subsequently these plants can be fed to animals and the vaccine is presented to and taken up by the mucosal surfaces in the intestine, thus providing a direct feed-vaccination. In addition to the vaccine itself, substances that stimulate vaccine uptake and activity (so-called adjuvants) and the route of vaccine delivery (injection, inhalation, feed, etc.) are factors that are strongly investigated and further developed by biotechnological methods.

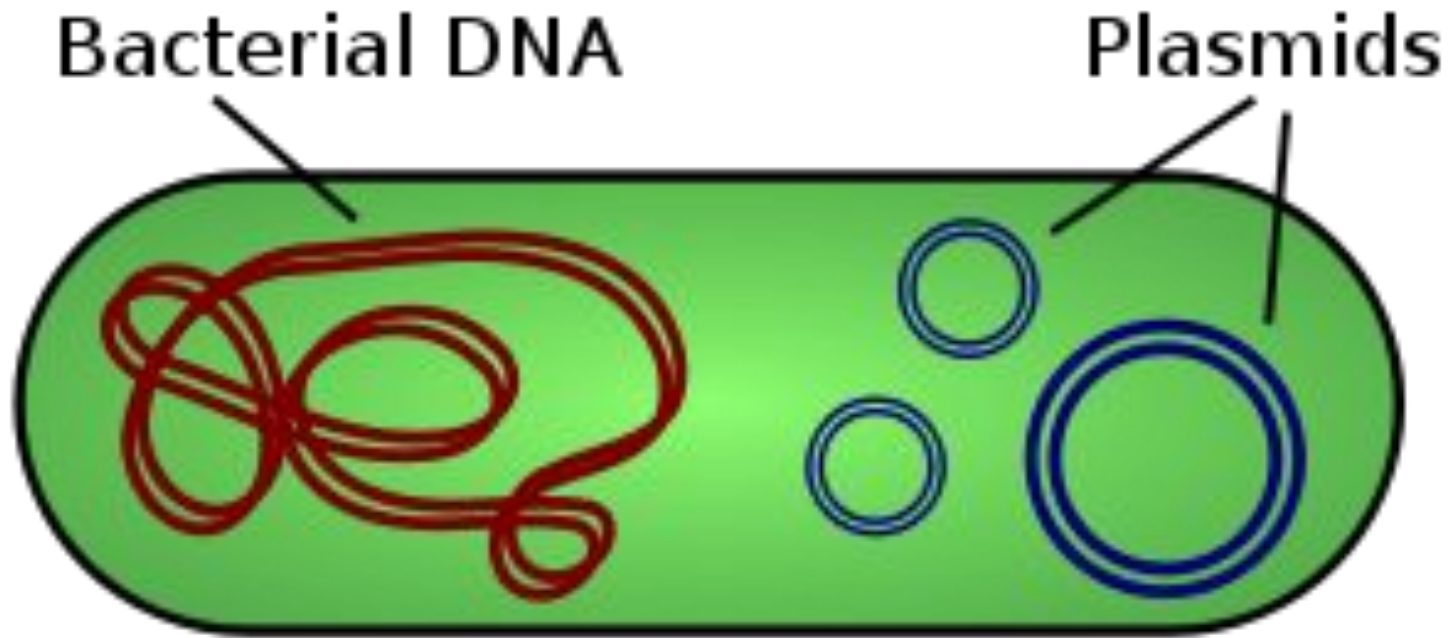
Recombinant bacterial vaccine

In medicine and veterinary variety of vaccines against infectious diseases of humans and animals are widely used. Unfortunately, a number of vaccines weakly immunogenic or have side effects, or require a high production costs.

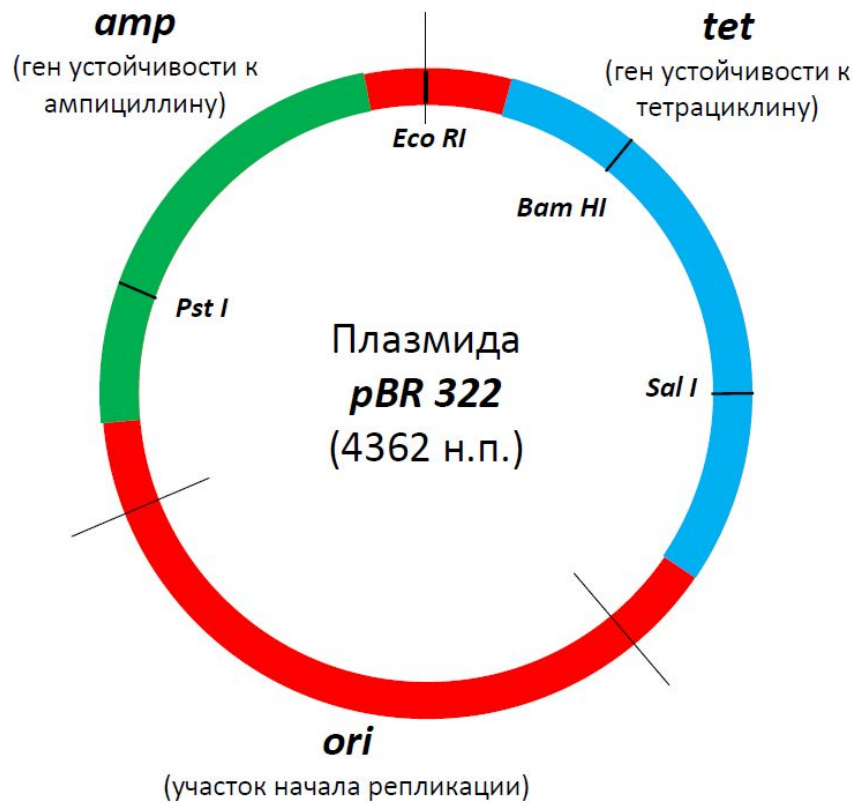
Immunogenic properties of pathogens are often determined by specific protein or polysaccharide molecule of the pathogen, which is encoded by a single gene. Current achievements of genetic engineering provide an opportunity to force prokaryotic or eukaryotic cells to synthesize specific antigen of the pathogen, which will serve as the basis for creating genetically engineered vaccine.

For the construction of live genetically engineered (recombinant) vaccine three of components are required:

1. ***Vector*** - the carrier of heterologous protective antigens;
2. ***Gene*** synthesising of heterologous antigen;
3. ***Genetic structures***, providing a stable and controlled expression of protective antigens that in its turn can induce an effective protection of the immunized organism.



Vectors - the common name applied to a DNA molecule derived from a plasmid or bacteriophage into which can be inserted or cloned DNA fragments; they contain one or more unique restriction sites for the incorporation of foreign DNA and being able to autonomous replication in a host, the intermediate body so that reproduce the sequence of the cloned structure.



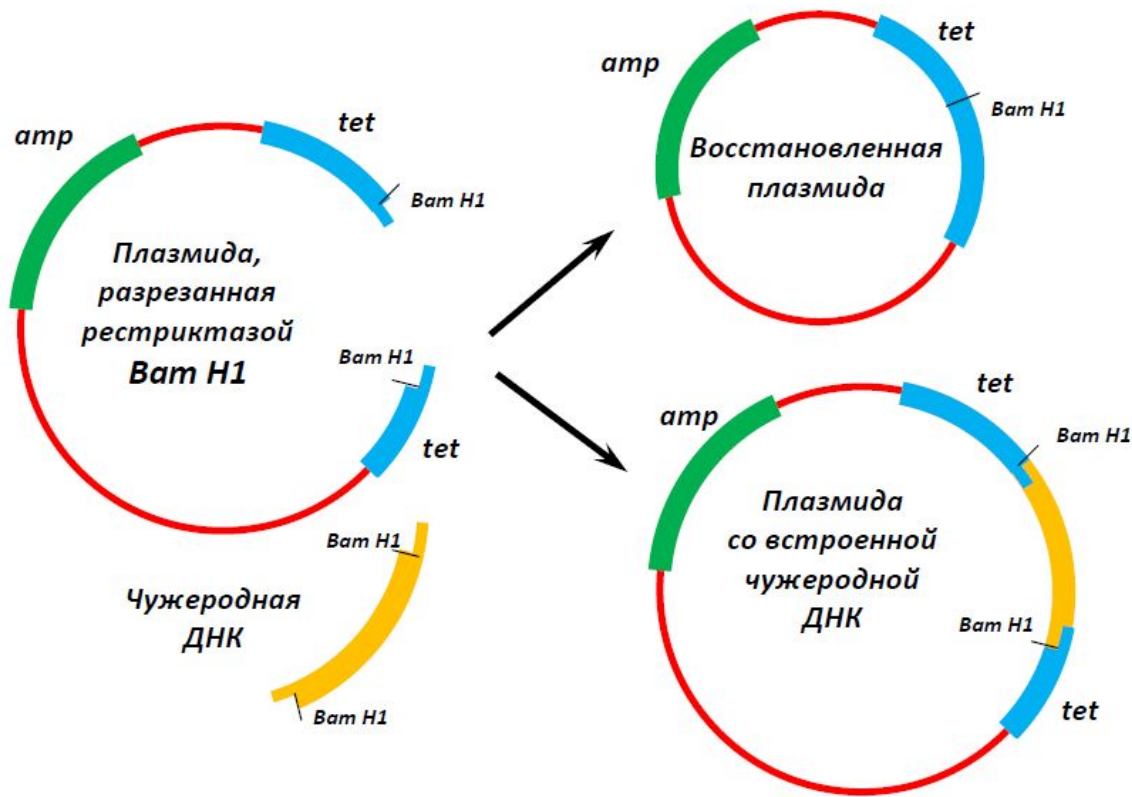
Plasmids are double-stranded and generally circular DNA sequences that are capable of automatically replicating in a host cell. Plasmid vectors minimalistically consist of an origin of replication that allows for semi-independent replication of the plasmid in the host.

Plasmids are found widely in many bacteria, for example in *Escherichia coli*, but may also be found in a few eukaryotes, for example in yeast such as *Saccharomyces cerevisiae*.

The first really useful plasmid for genetic engineering, pBR322, was pieced together by Francisco Bolivar, and others in Herbert Boyer's laboratory in the 1970s. What makes pBR322 useful is that it contains an **ampicillin resistance gene** and a **tetracycline resistance gene**.

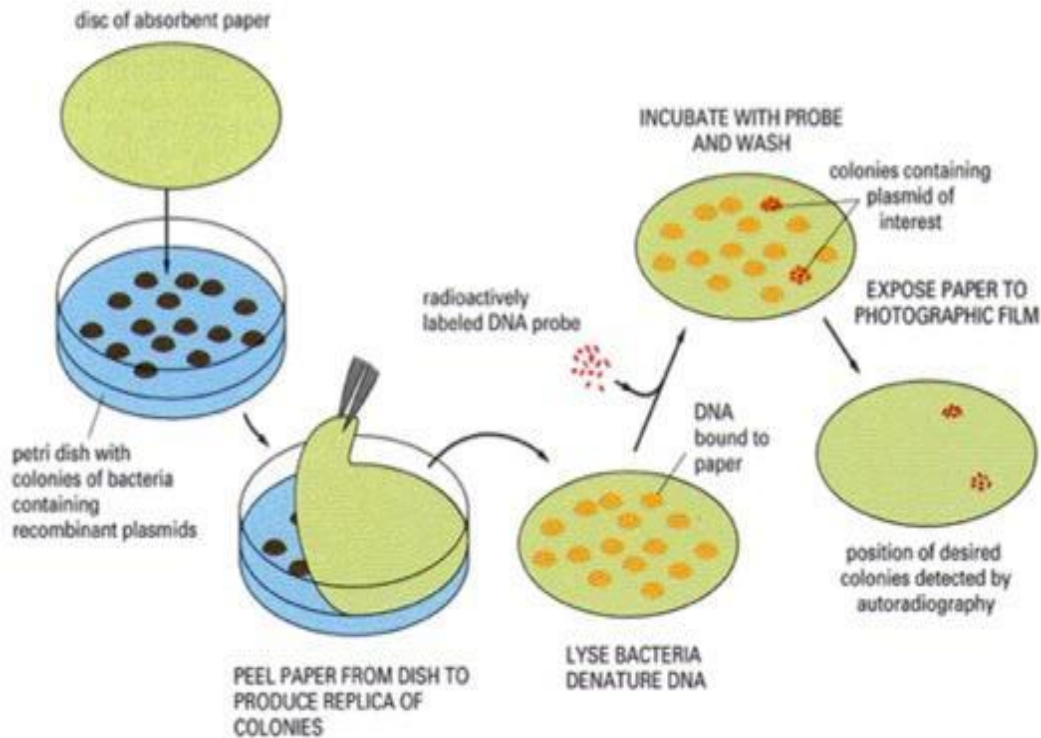
In addition it has a relaxed **origin of replication** and accumulates to high numbers in *E. coli*. Its entire 4363 base-pair sequence has been determined, and 21 common enzymes are available that recognize only a single site within it.

The plasmid also contains the restriction sites **Pst I**, **Bam HI** and **Sal I**, moreover a first stored in the **amp gene**, and the other two - in the **tet gene**. This is an important factor helping to modify the plasmid.



Assume that fragment, which had previously been cut from other DNA with restriction enzyme Bam HI (i.e. at the ends it has nucleotide sequence characteristic for restriction site Bam HI), must be incorporated into a plasmid. For this purpose the plasmid is treated with Bam HI, (which cuts circular molecule in the restriction site and forms a linear DNA segment) and then portions of the foreign DNA are added. Since at the end of the DNA fragments complementary nucleotide sequences are located, they will start to "stick together", with two variants of splicing.

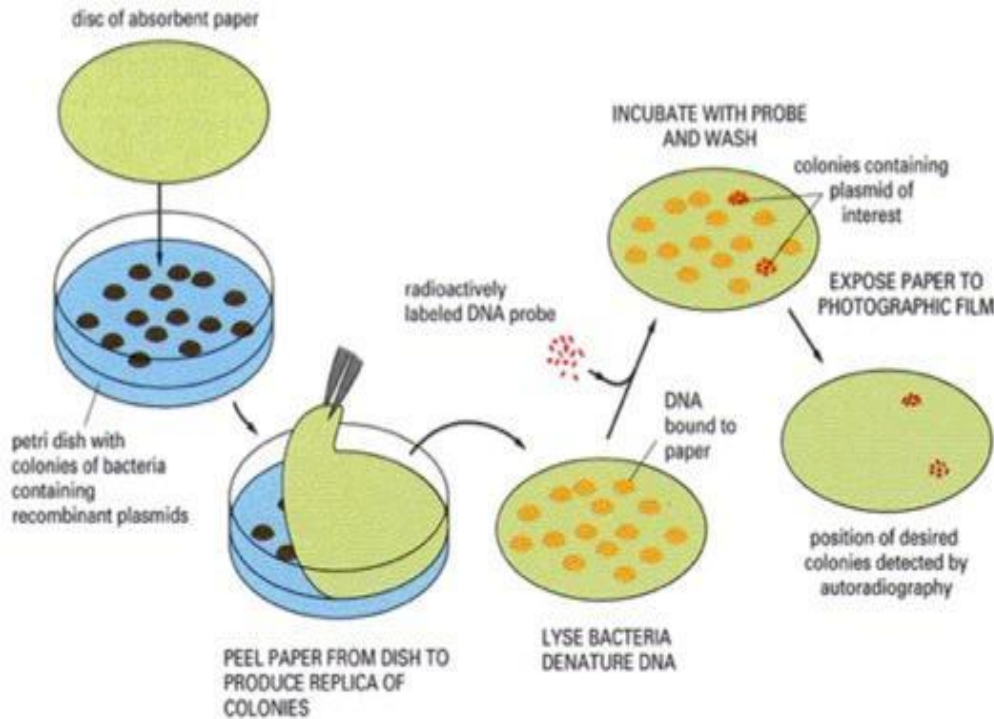
Plasmids are introduced into *E. coli* cells. For this, the cells are treated by ions Ca^{2+} , making their membranes permeable to DNA. The resulting bacteria are plated on medium containing ampicillin. In this environment, colonies of bacteria containing plasmids will grow, the other colonies are inhibited. On this basis one can distinguish the bacteria containing the plasmid; Colonies containing the plasmid are reprinted on medium containing tetracycline. As the foreign DNA is wedged into the gene *tet*, deactivating it, bacterial colonies with modified plasmids are inhibited by tetracycline. Thus they are visually distinguishable from bacterial colonies with restored plasmids. Thus, *E. coli* colonies are isolated, in the plasmid of which foreign DNA is integrated. They are seeded in a normal medium for further cloning.



Replica Plating for Colony Screening

We will now examine how to select for transformed cells that harbor the plasmid of interest if that plasmid contains genes for antibiotic resistance. Think back to the plasmid pBR322. The original plasmid contains genes for resistance to ampicillin and tetracycline. Cells transformed with the original plasmid will be resistant to both of these antibiotics. If we cut the plasmid with *Bam*H1, and insert our new gene at this site we will interrupt the gene for tetracycline resistance. If we transform cells with this new construct, the cells will not be resistant to tetracycline, but they will still be resistant to ampicillin. **Procedure:**

Spread about 10 000000 bacteria from the transformation reaction mixture on the surface of a plate containing nutrient agar .Incubate the plates overnight at 37o C to allow the cells to grow as colonies. Press a piece of velvet against the surface to pick up some of the cells.

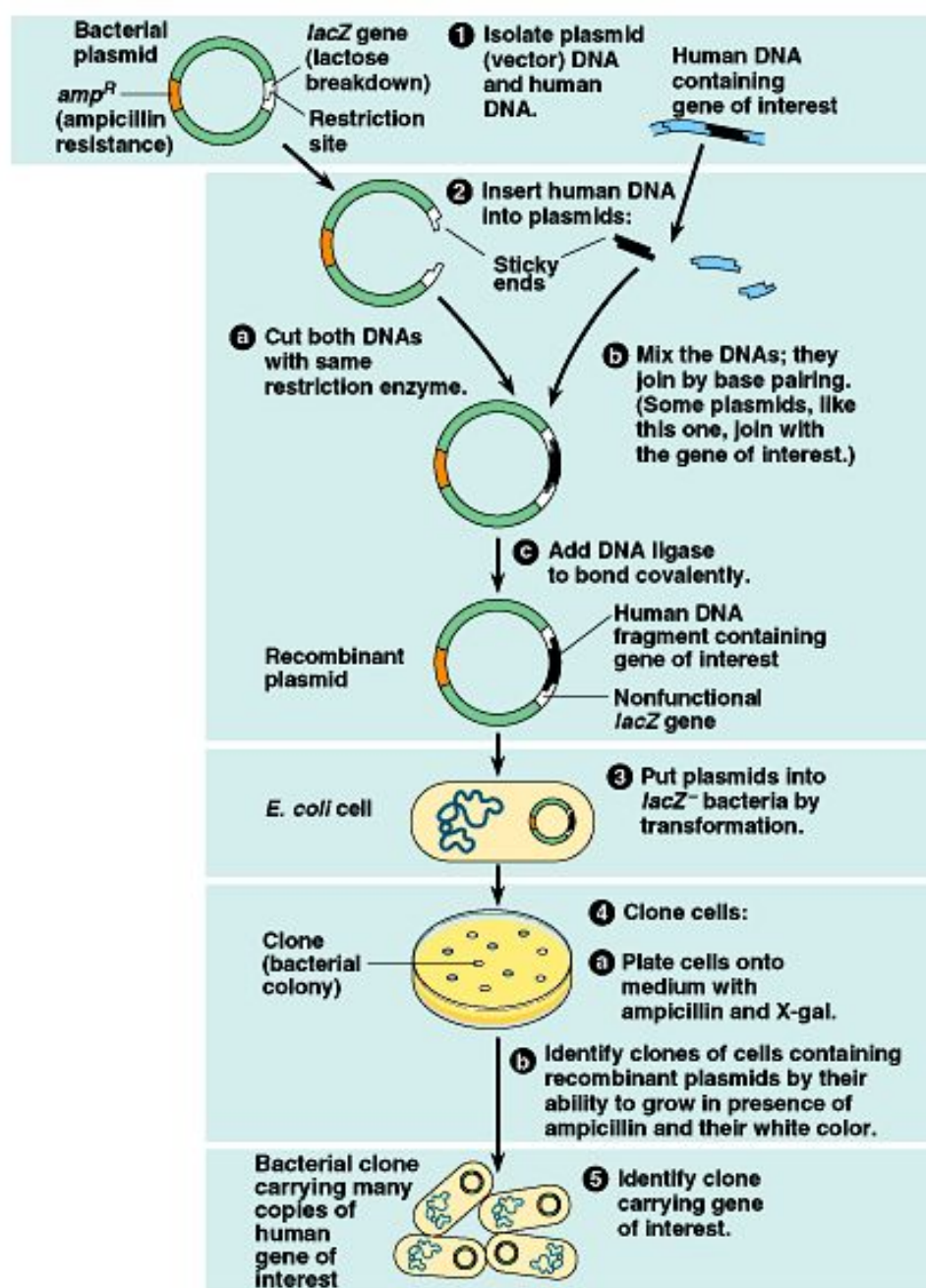


Transfer to new plates containing normal media or medium containing antibiotics and grow overnight.

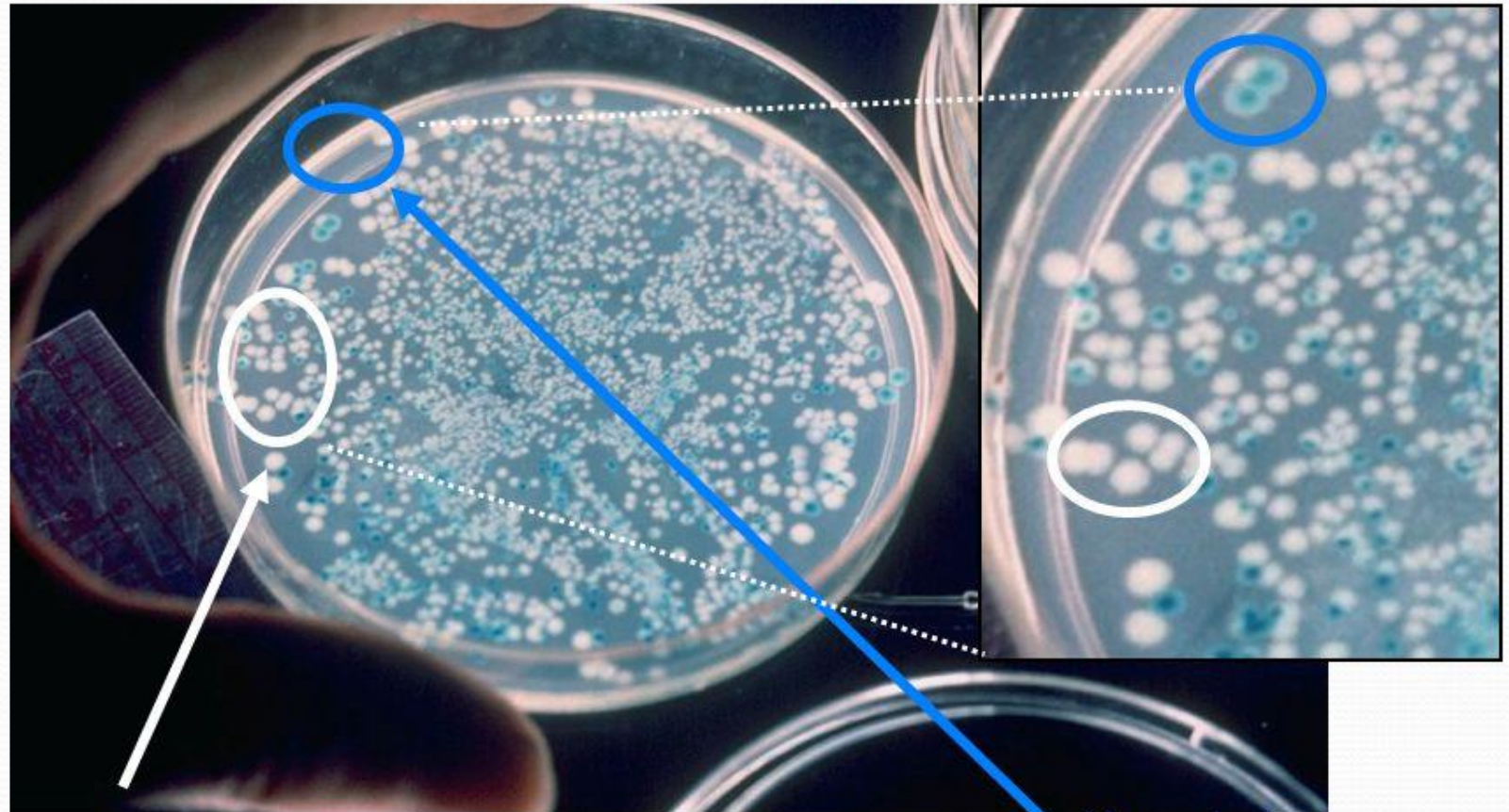
Expected Result: The colonies of cells pressed onto normal medium will grow vigorously as before and they represent the total number of cells that we plated.

In our example, cells plated on medium containing ampicillin will not grow unless they were transformed with either the original plasmid or the plasmid containing the inserted gene. The number of colonies tells us about the efficiency of transformation.

Select colonies of cells containing the desired transformation. Cells growing on medium containing both ampicillin and tetracycline must be those cells that were only transformed with the original plasmid and not the plasmid containing the gene we want. We now must go back to the ampicillin plate and select and save those few colonies that were unable to grow in the presence of tetracycline. These are the cells that we will save and grow and which contain the desired plasmid.



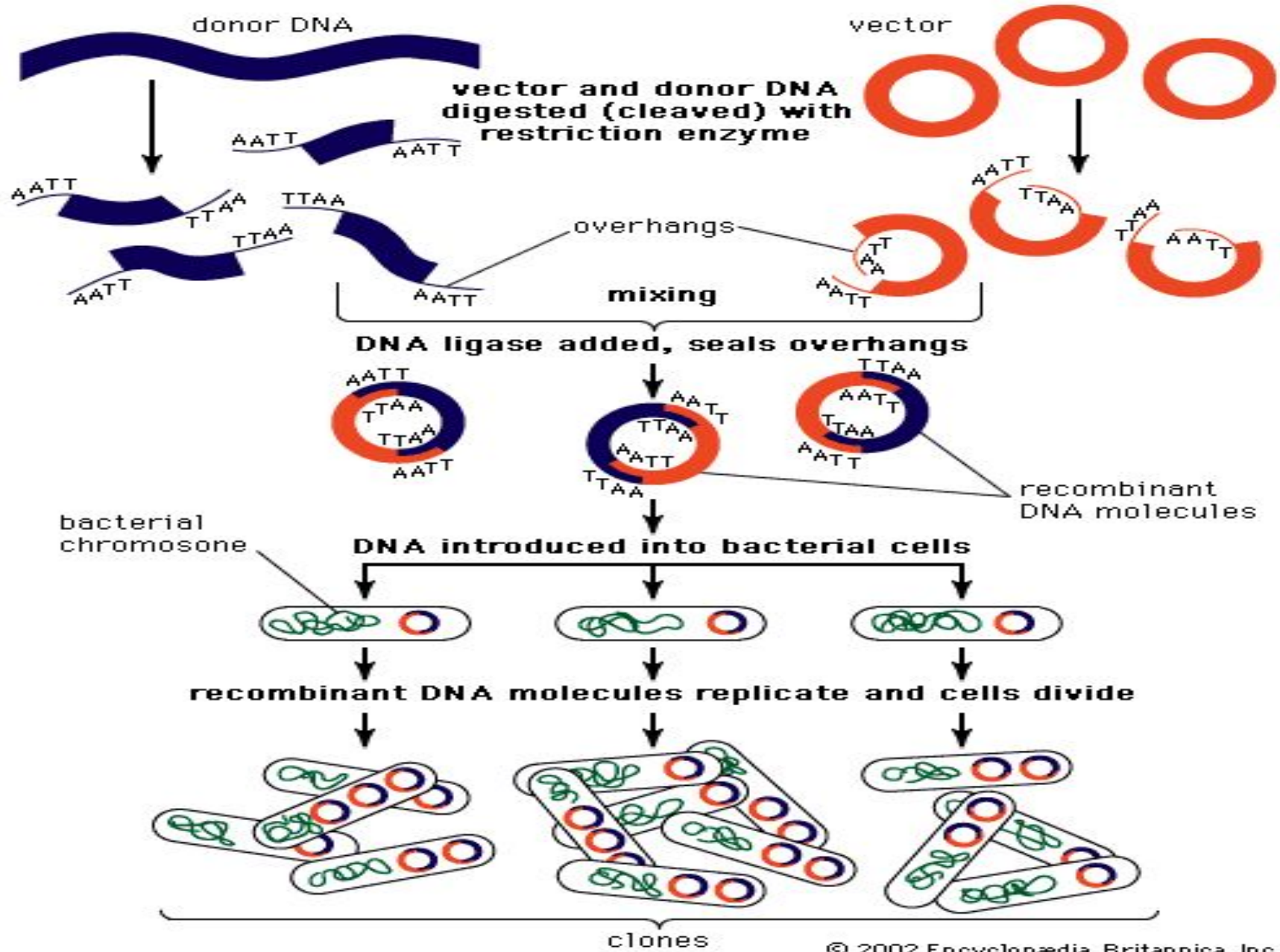
Blue/white screening (Bacterial colonies transformed with pUC18)

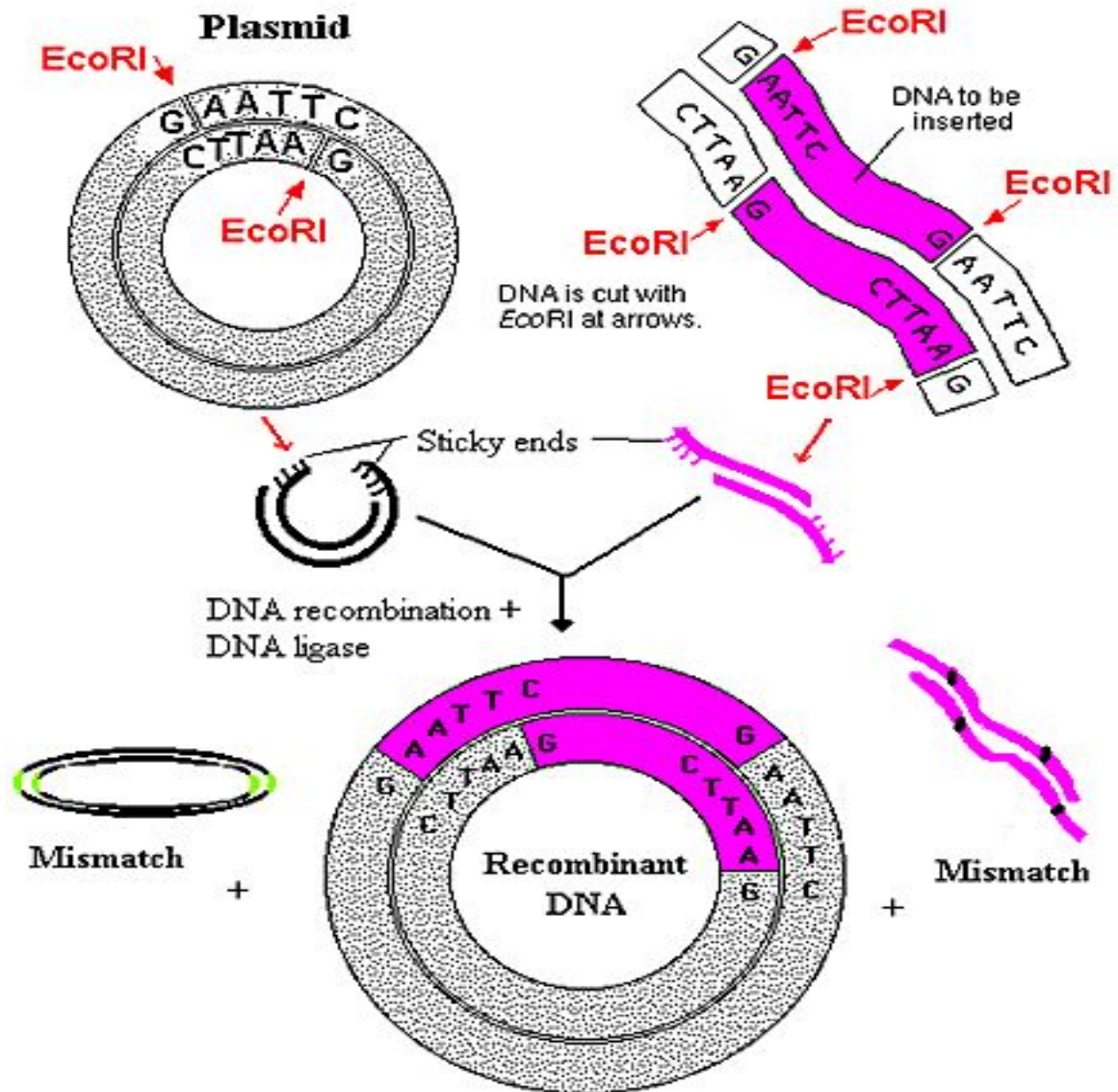


White colonies
(contain recombinant DNA
molecules)

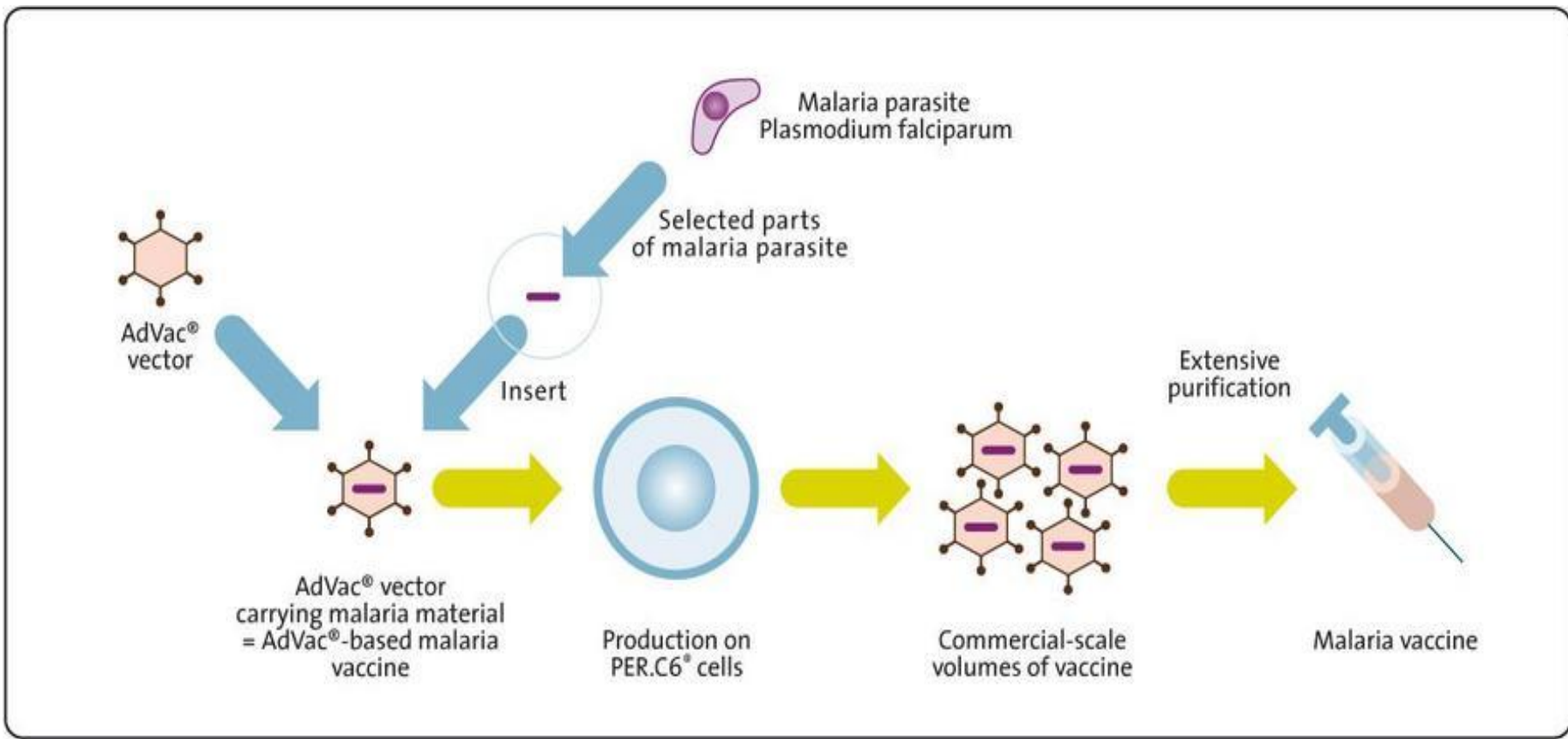
blue colonies
(contain non-recombinant
DNA molecules)



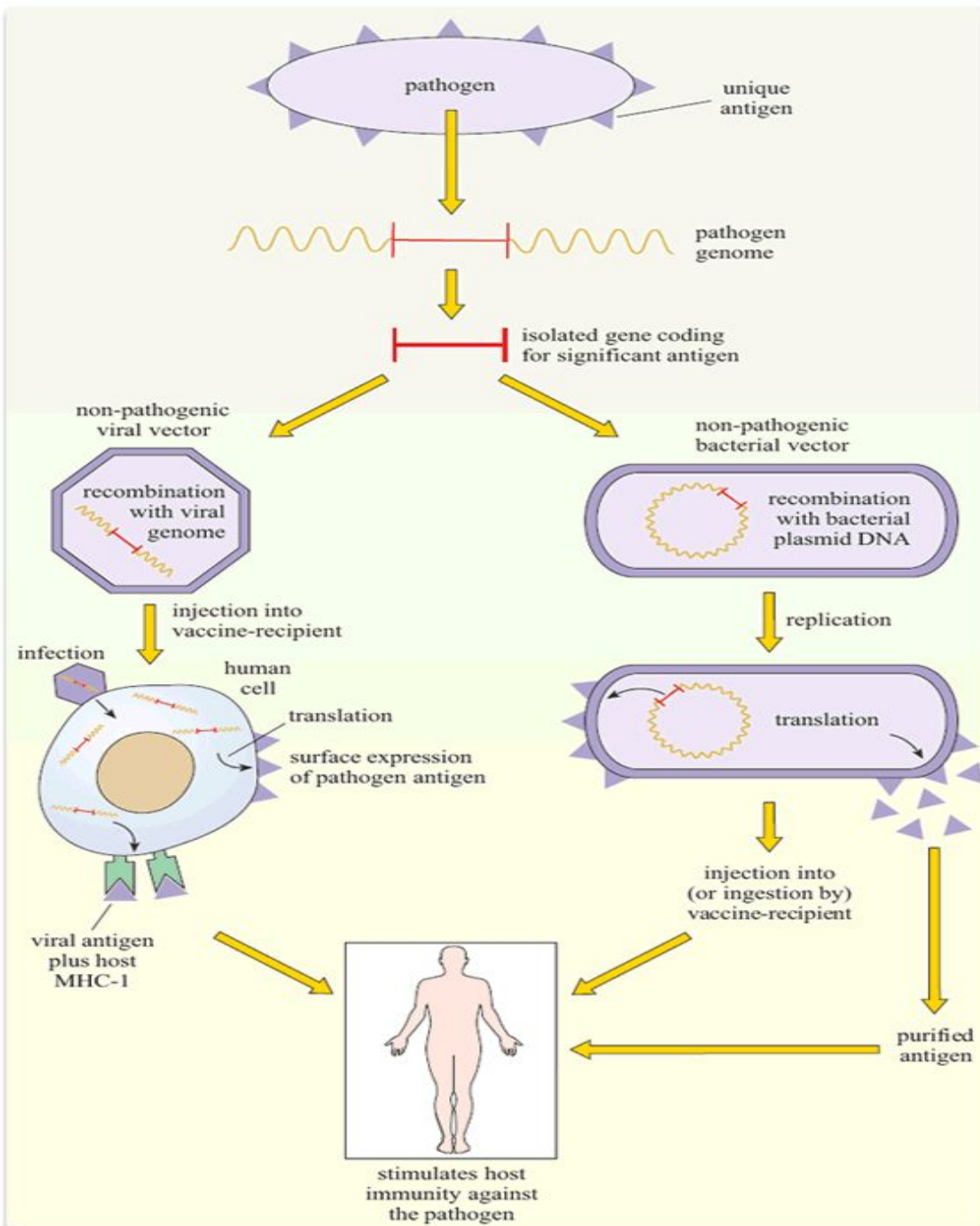




Inserting a DNA Sample into a Plasmid

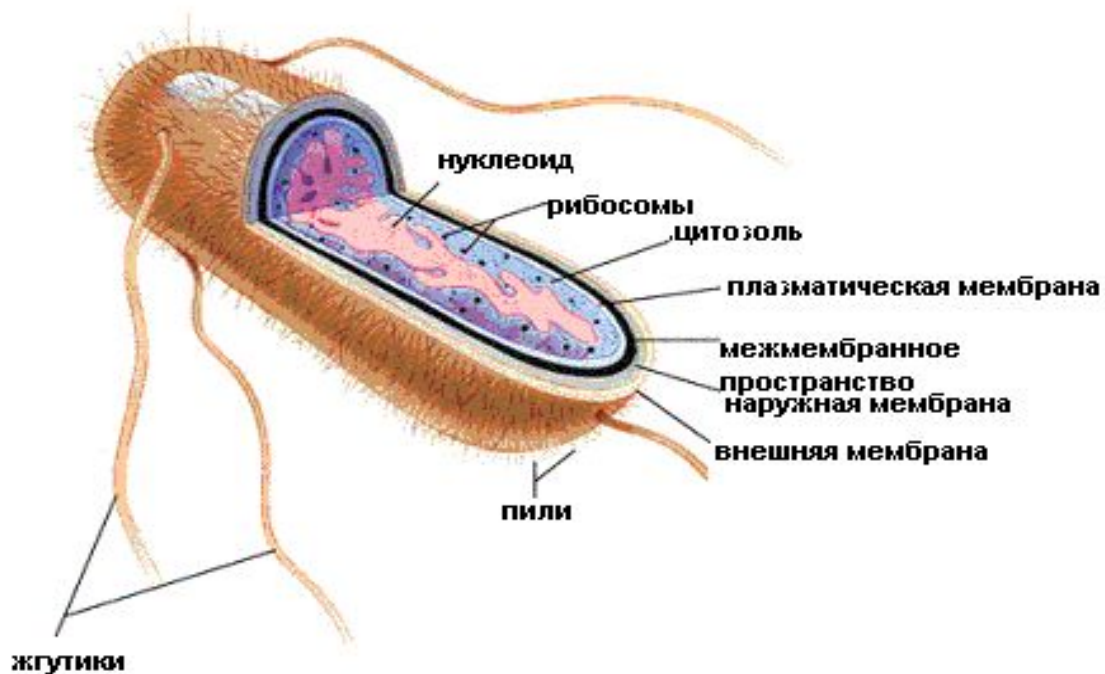


Anti-viral vaccines have become the main object of the application for genetic engineering due to simplicity of the organization of viral genomes. A more complicated structure of the bacterial cells and relatively low cost of antibacterial vaccines are factors hindering the development of genetic engineering. In the future, vectors, in which are integrated not only the genes that control the synthesis of pathogen antigens, but also genes encoding various mediators of (proteins) immune response (interferons, interleukins, etc.) will be used.

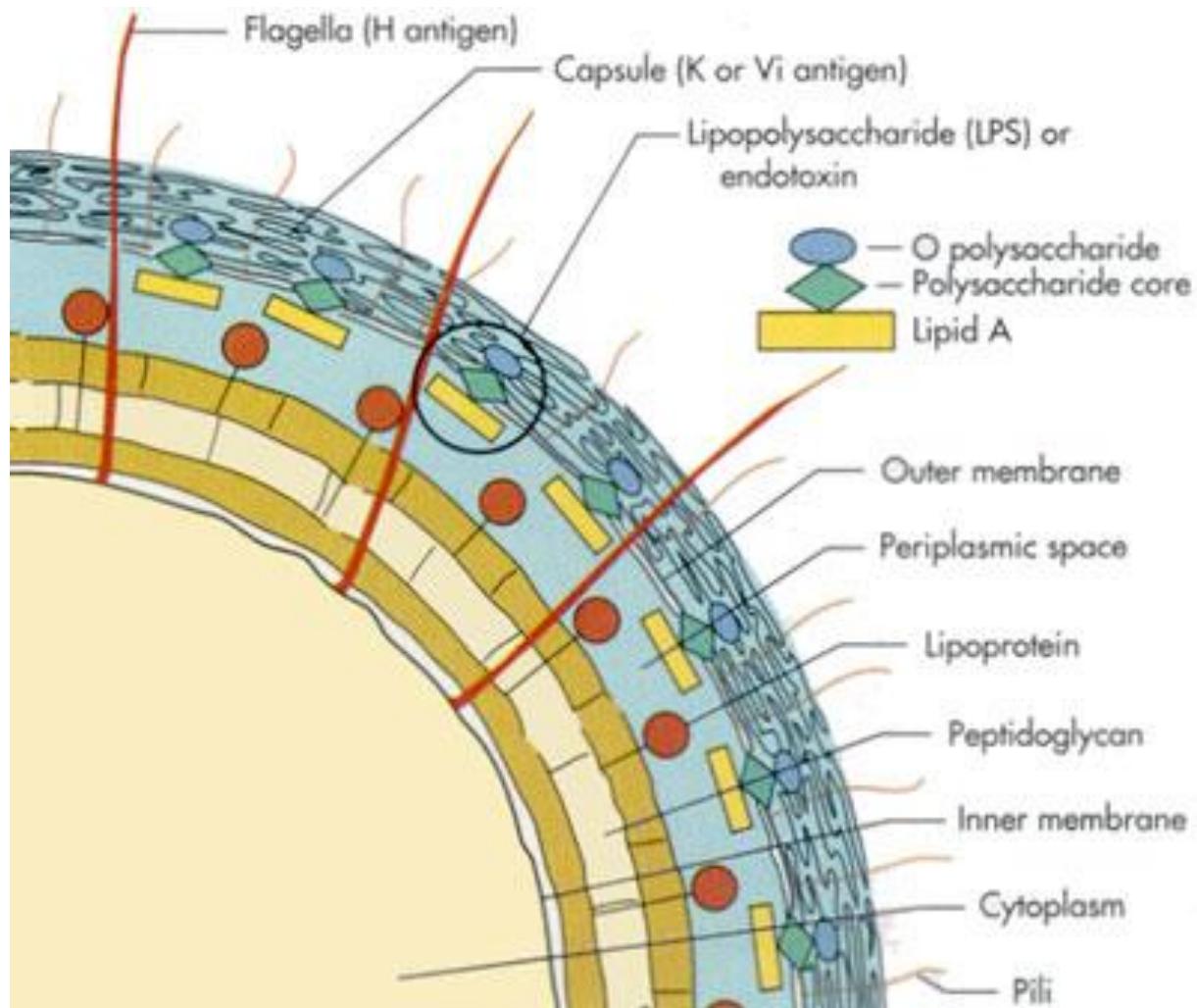


Treponema pallidum - causative agent of syphilis, was the first bacterium which attracted attention of researchers involved in the creation of genetically engineered vaccines. And this is no accident. First, despite the fact that at the disposal of modern medicine there are effective methods of diagnosis and therapy, syphilis acquired epidemic spread in both developed and developing countries, and secondly, obtaining pure cultures of *Treponema pallidum* is very difficult, because it is not growing in artificial medium and, thirdly, it is impossible to get the vaccine against it by conventional methods based on the extraction and purification of antigens. Lovett and colleagues (University of California) cloned DNA of this spirochete into *E. coli* cells using bacteriophage as a vector.

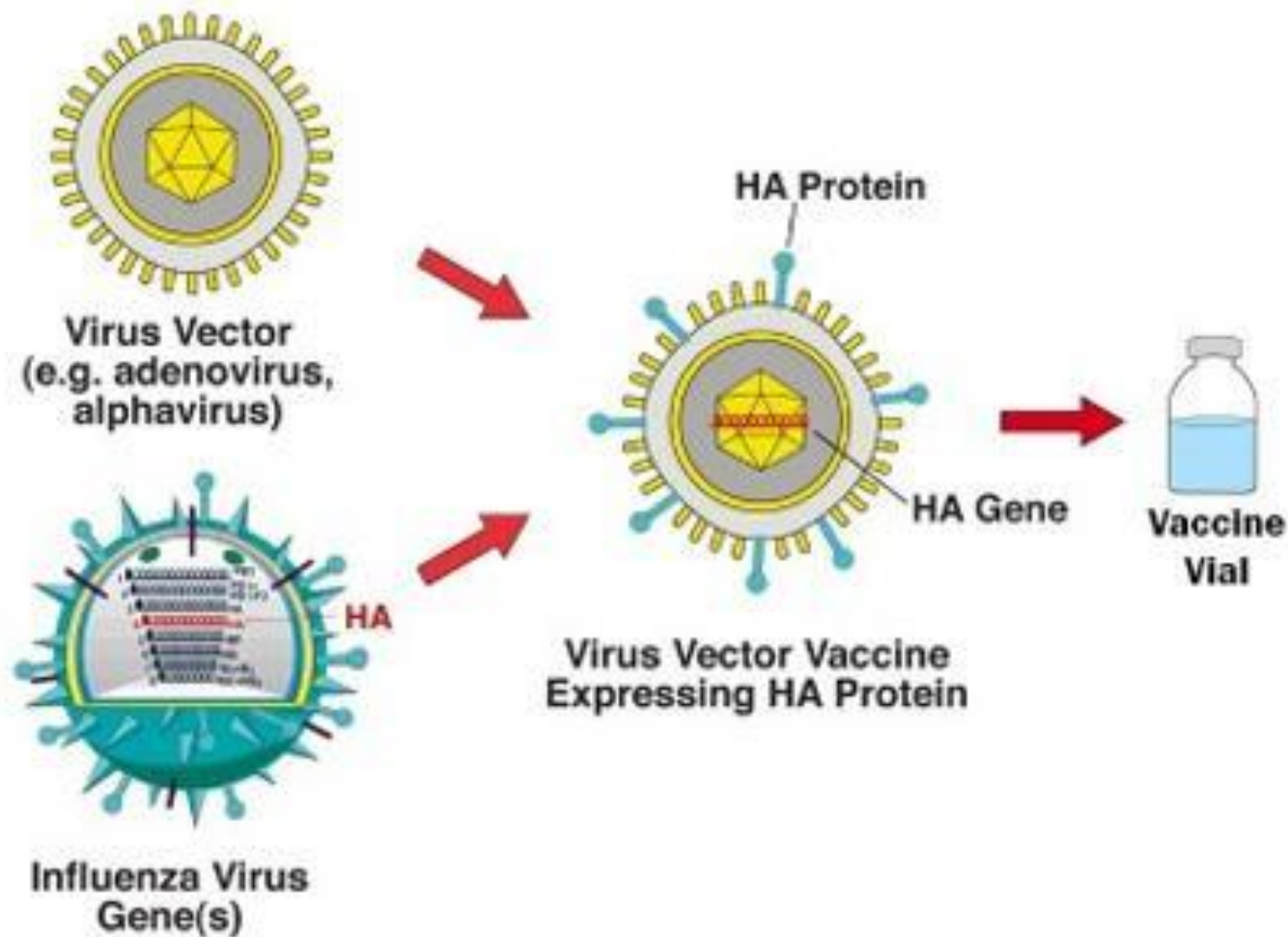
The genetic material for the experiment was isolated from the testicles of specifically infected rabbits. They got the strain *E. coli*, which contained at least seven specific antigens of spirochete. These studies were used to develop more specific diagnostic tests for syphilis and the production of effective vaccine.



In veterinary medicine, the first genetically engineered antibacterial vaccine which found application in practice was a vaccine against *Colibacillosis (Escherichiosis)* of pigs and calves caused by pathogenic strains of *E.coli*. The developer of the vaccine is Dutch Veterinary Pharmaceutical Company "Intervet international". In order to isolate the protein in sufficient quantities for a preparation of a vaccine, they cloned the gene responsible for the synthesis of adhesion antigens of *Escherichia coli* K88 and K99, in a strain of *E. coli* K-12. These antigens in combination with an adjuvant were used to obtain the vaccine. Immunization of cows and pigs with vaccine causes the formation of protective antibodies, which are then transmitted to the newborn with colostrum and milk. Similar vaccines have been also developed by company "Cetus" together with "Norden laboratories" (USA) and "Tech America Group".



To antigens, providing full protection can be attributed adhesion antigens of pathogenic *Escherichia coli* and heat labile enterotoxin O-and Vi-antigens of *Salmonella*, Cholera and Diphtheria toxins, pathogens toxins of tetanus, botulism, gas gangrene, malignant edema, capsular antigens Plague and others.

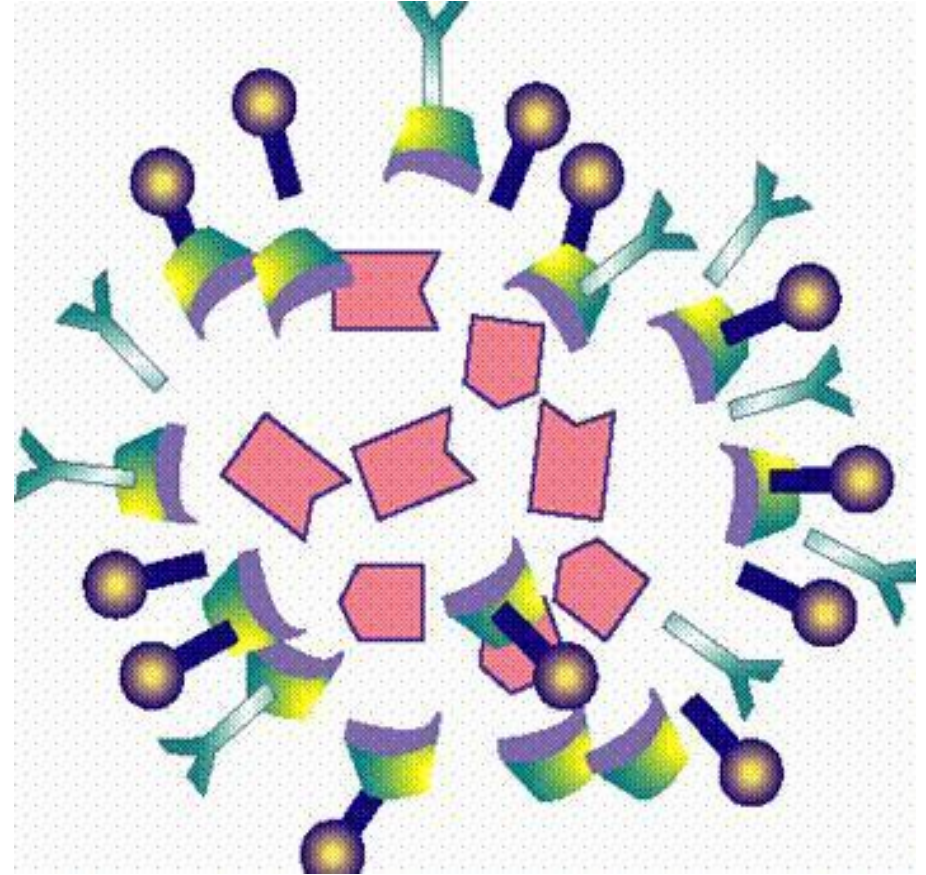
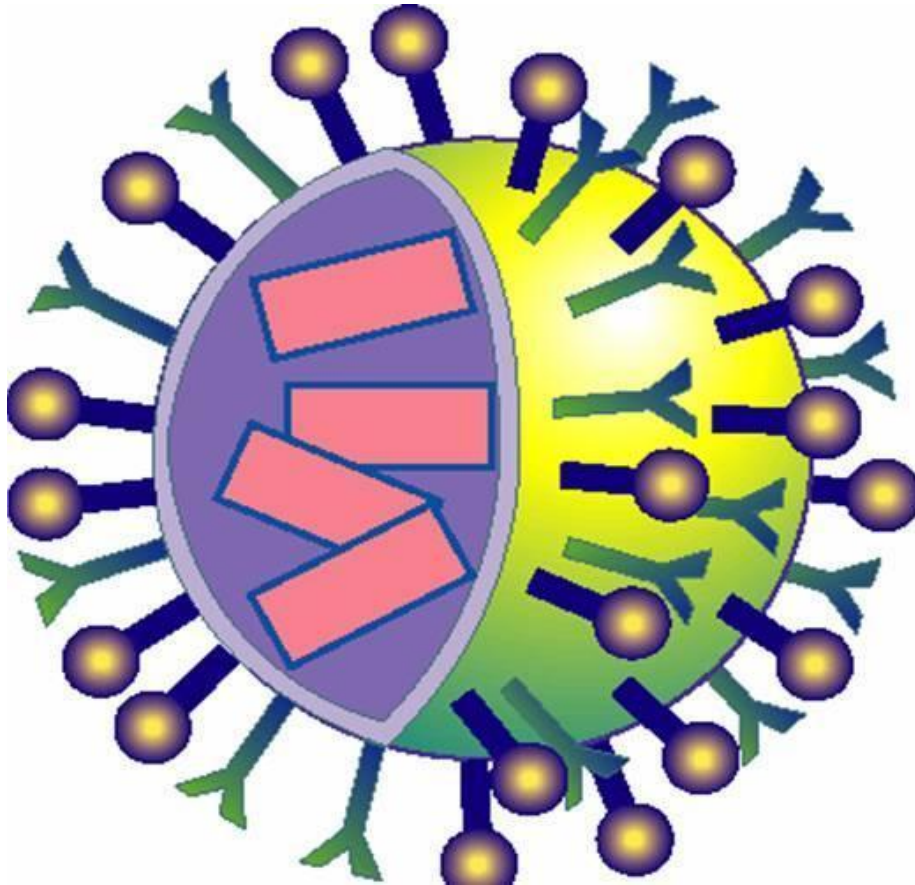


Vector" vaccine on the basis of viruses. A live cowpox virus (CPV) belonging to the genus of Poxviruses was used as an effective smallpox vaccine. DNA of CPV replicates in the cytoplasm of infected cells, but not in nucleus due to the presence in virus of genes of DNA polymerase, RNA polymerase and enzymes performing capping, methylation and polyadenylation of mRNA. Therefore, if in genome of CPV integrate foreign gene so that it will be under the control of CPV -promoter, it will be expressed independently of the regulatory and enzyme systems of the host. CPV has a wide range of hosts and it remains viable for many years after lyophilization does not possess oncogenic properties, and therefore can be used to create so-called vector vaccines.

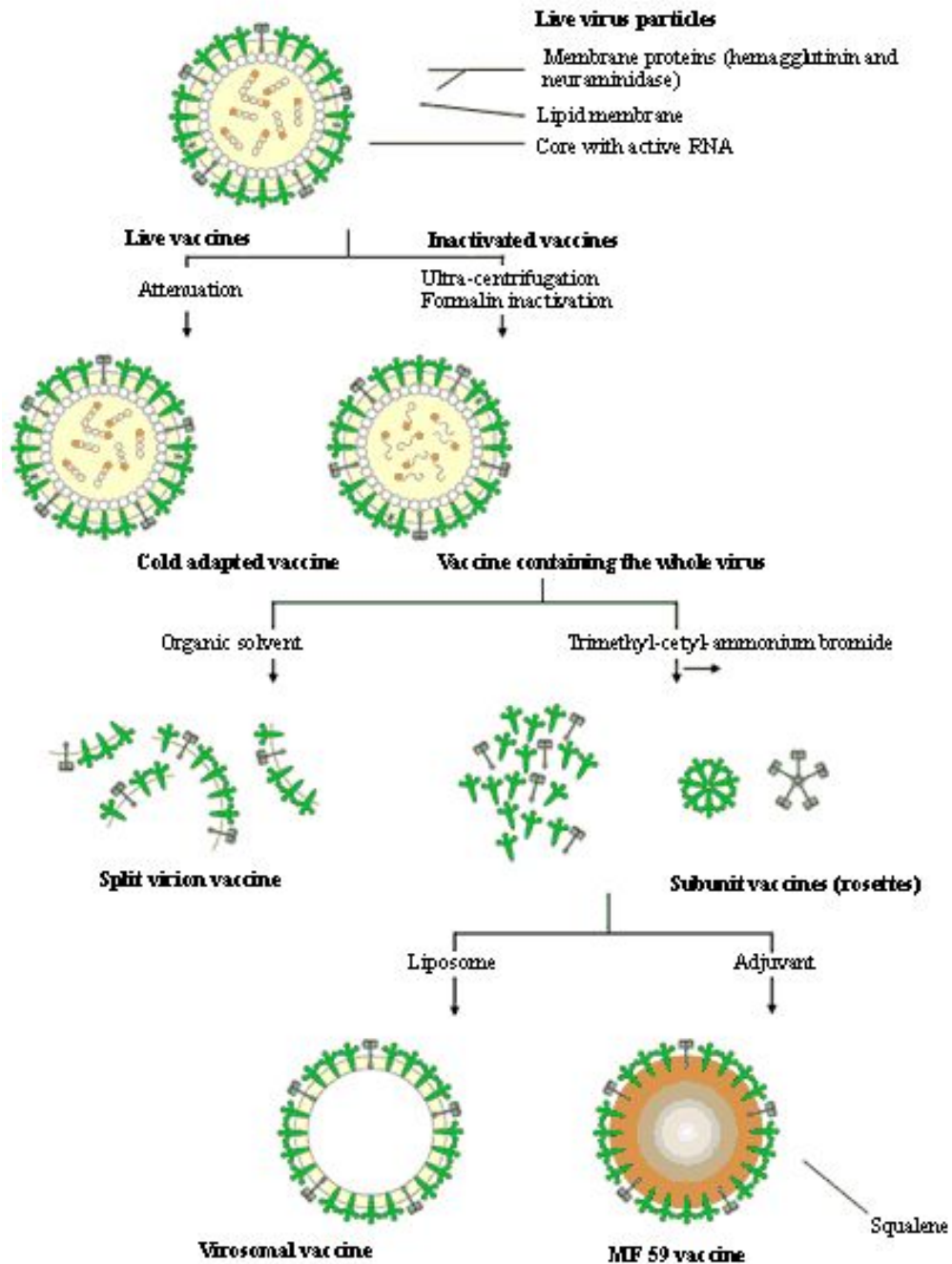
Live recombinant **virus vaccine has several advantages** over non-living virus and subunit vaccines:

- 1) presentation of authentic antigen does not differ from that in normal infection,
- 2) virus can replicate in the host cell and increase the amount of antigen that activates the production of antibodies by B-lymphocytes (humoral immunity) and stimulates the production of T-cells (cellular immunity),
- 3) **The lack of a live recombinant virus vaccine** is that in vaccinated individuals with reduced immune status (such as AIDS) severe viral infection may occur. Gene, encoding human interleukin-2, which stimulates T-cell response and limits the proliferation of the virus, may be inserted into a viral vector to solve this issue.

Subunit vaccines



Vaccines containing only the individual components of the pathogen are also called as "*subunit*". Subunit vaccines have their own advantages and disadvantages.



The advantages are that the preparation containing the purified protein is immunogenic, stable and secure, its chemical properties are known, there are no additional proteins and nucleic acids, which could cause undesired side effects in the host organism.

The disadvantages are that cleaning of a specific protein is expensive, and conformation isolated protein can be different from the one it has *in situ* (ie, in the composition of viral capsid or envelope), which may lead to a change in its antigenic properties. Decision on the production of subunit vaccines are made taking into account all relevant to the case of biological and economic factors.

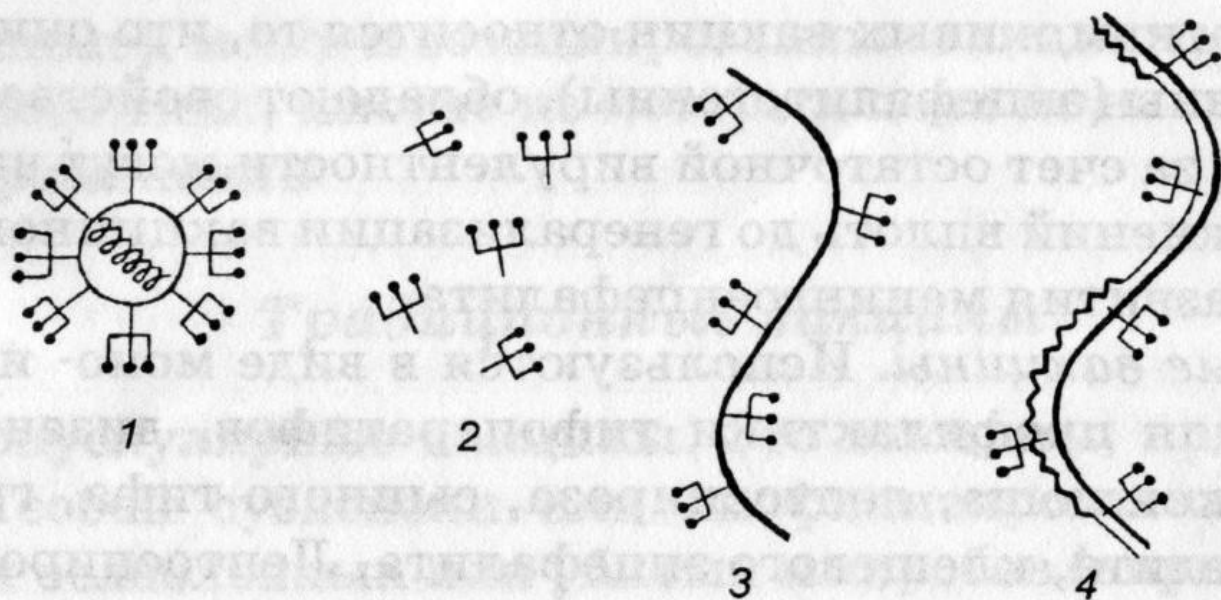
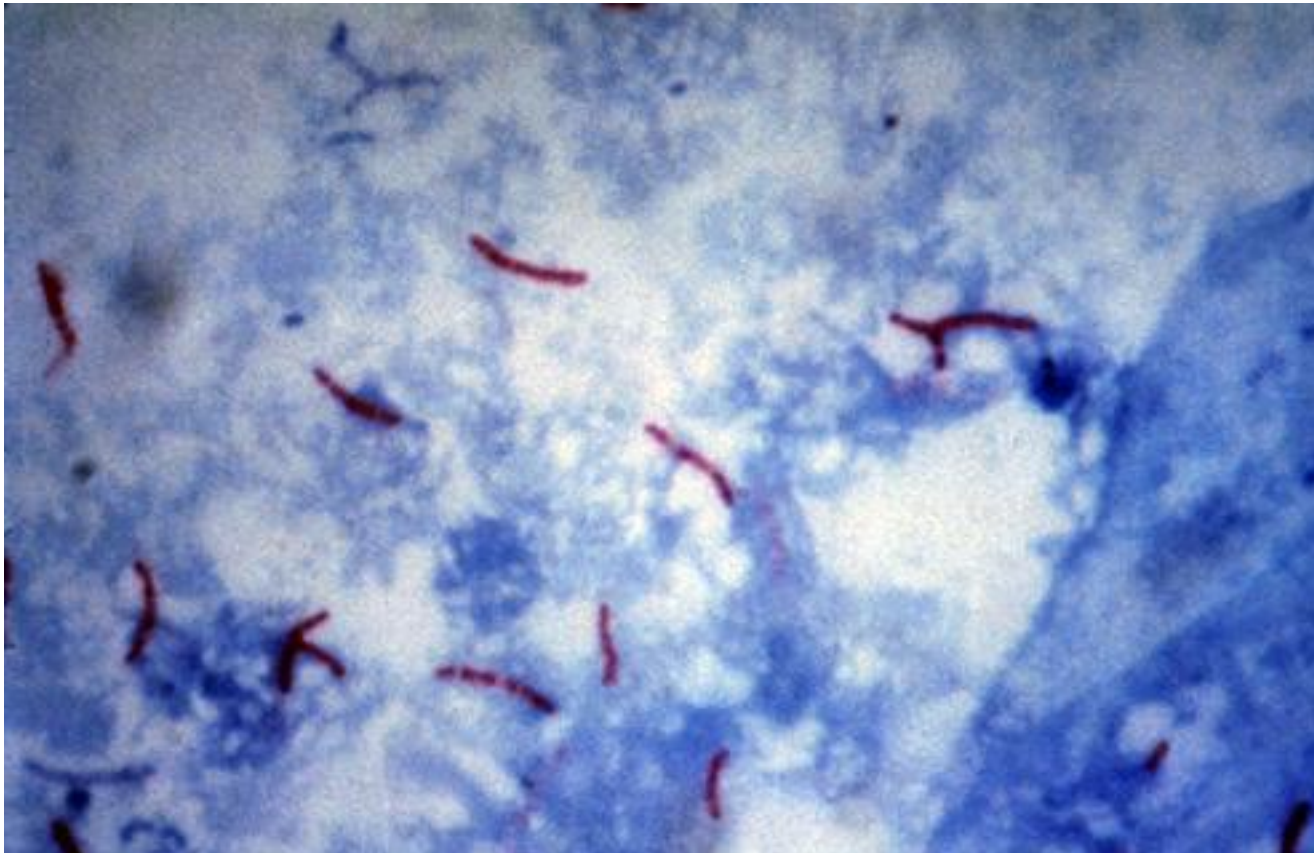


Рис. 69. Принцип конструирования субъединичных и синтетических вакцин:

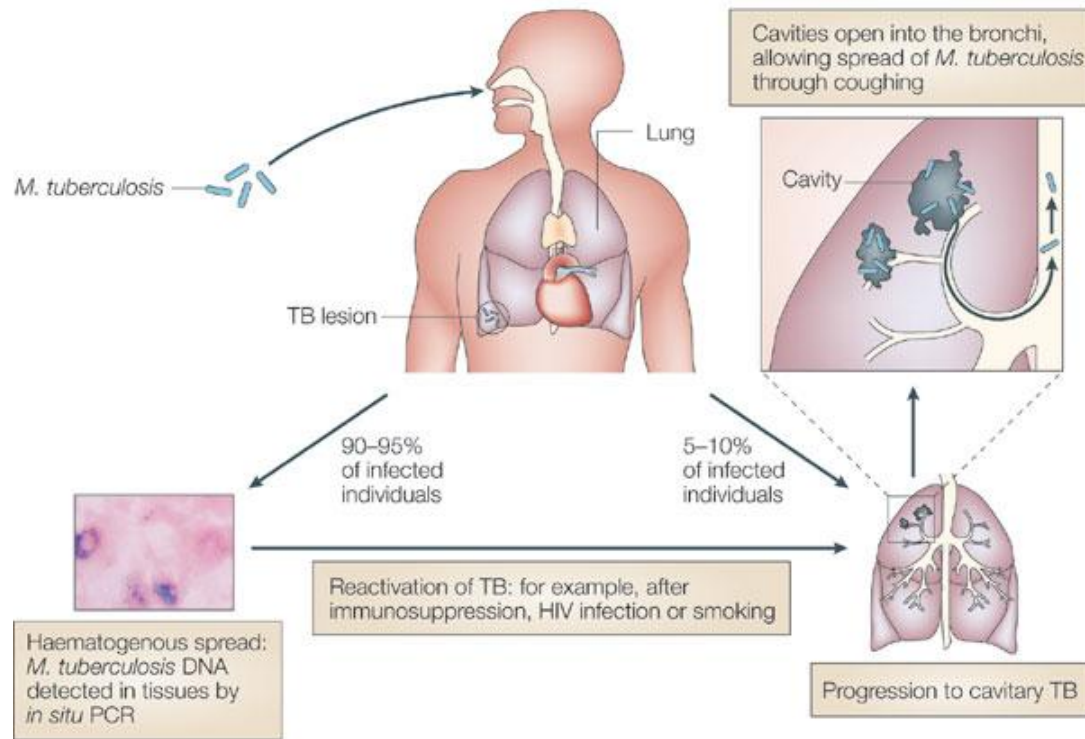
1 – вирион; **2** – субъединичная вакцина без носителя; **3** – субъединичная вакцина с носителем; **4** – субъединичная вакцина с носителем и иммуностимулятором



In veterinary science, some progress has been made in the development of subunit **vaccines against FMD**. Vaccine containing a virus inactivated with formalin is used to protect animals against this infection. World annual production of the vaccine is approximately 1 billion doses. The main antigenic determinant inducing antibody formation, is a viral capsid protein 1 (**VP1, viral protein 1**). This is a weaker antigen than intact viral particles, but it induces the formation of antibodies and protect animals against infection. Therefore, attempts to clone VPI-gene were made.



Recently **heat shock proteins** of *Mycobacterium tuberculosis*, as the basis for a subunit TB vaccine have been intensively studied. Using ELISA and monoclonal antibodies to HSP65 the presence of heat shock proteins of *Mycobacterium tuberculosis* in sera of patients with confirmed tuberculosis and in sera of patients with suspected tuberculosis was defined (I.A.Basnakyan et al, 2010). HSP65 *Mycobacterium tuberculosis* was detected in cerebrospinal fluid of patients with tuberculous meningitis, and the presence of this antigen may be a diagnostic marker for tuberculous meningitis. The presence of serum antibodies to HSP70, HSP65 and HSP 16 *Mycobacterium tuberculosis* in tuberculosis were investigated. Significantly higher levels of antibodies were found in the sera of patients with tuberculosis than in blood serum of healthy people, thus proving important role of heat shock proteins in the stimulation of immunity.



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The methods for production of **recombinant heat shock protein HSP70M** of *Mycobacterium tuberculosis*, its properties and analysis on conformity to requirements of preclinical trials were studied by A. Sharapova et al. (2009). Szewczuk YS et al. (2009) found that **recombinant HSP70M *Mycobacterium tuberculosis* increases the activation of innate and adaptive immunity in the case of combined administration with bacterial antigens.** In an effort to create a safer and more effective TB subunit vaccine immune protective properties of purified extracellular proteins of *M. tuberculosis* were studied. From liquid bacterial cultures six major secreted proteins out of 100 were isolated and purified. Each of them separately, and then in various combinations were used for immunization of guinea pigs. Animals were administered by aerosol approximately with 200 living cells of *M. tuberculosis*, that is extremely high dose for them. After 9-10 weeks animals were killed and their lungs and spleen were examined for the presence of pathogenic bacteria. At the introduction of some combination of purified proteins weight loss, lung and spleen damages, mortality rate were the same as in the case of vaccination with live BCG vaccine.

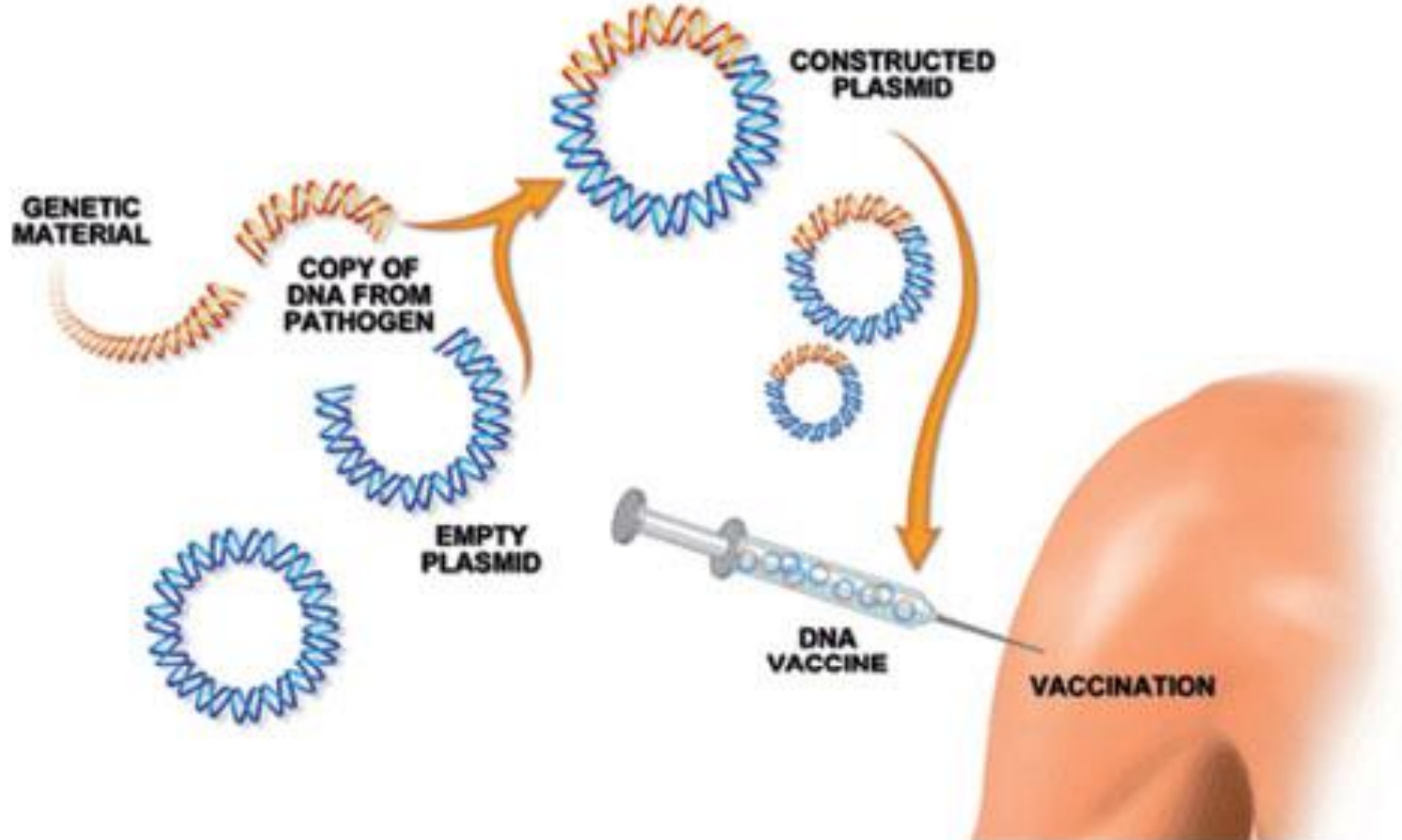
Bacteria as antigen delivery system.



Antigens located on the outer surface of bacterial cells are more highly immunogenic than which are localized in the cytoplasm. Therefore, one of the approaches used for the development of vaccines, is to place the protective antigen of pathogenic bacteria on the surface of living non-pathogenic bacteria. Many bacteria have flagella composed of flagellin protein, under a microscope, they look like threads extending from the bacterial cell. **If do so that flagella of nonpathogenic microorganism will bear a specific epitope of a pathogen, it can induce the production of protective antibodies.** This is the approach used when creating vaccines.



Synthetic oligonucleotide encoding the epitope of **cholera toxin subunit B** was inserted into hypervariable region of **Salmonella flagellin gene** and the resulting structure introduced in **flagellin-defective strain of Salmonella**. It was known that the epitope comprising 50-64th amino acid residues of B cholera toxin, induces the production of antibodies to intact cholera toxin. Chimeric flagellin functioned normally, and cholera toxin epitope was located on the surface of flagella. Immunization of mice with intraperitoneal injection of approximately 5 mln. live or killed bacteria with modified flagellin induced the production of large amounts of antibodies to a peptide (amino acid residues 50-64) and to the molecule intact cholera toxin. Similarly it is possible to integrate two or even three different epitope in one flagellin gene of *Salmonella* and create antibacterial polyvalent vaccine.



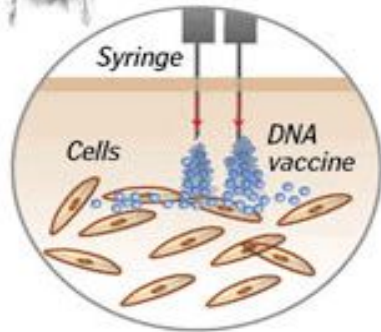
Schematic showing the injection of Vical's DNA vaccine technology (Source: Vijay Samant, PhD)

DNA vaccines. A new approach for inducing body's immune response without antigen injection is based on inclusion a target gene encoding a protein antigen into animal cells. In the first experiments of this kind *E. coli*-plasmid containing the cloned gene of protein-antigen, transcription of which was under the control of the promoter of animal viruses were conjugated to gold microparticles and mouse ear cells were bombarded with them.

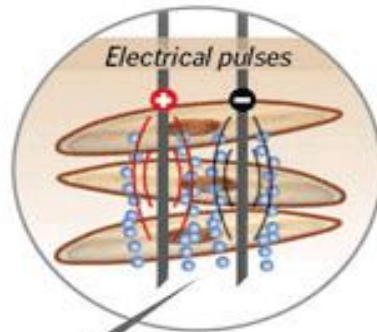


Detail

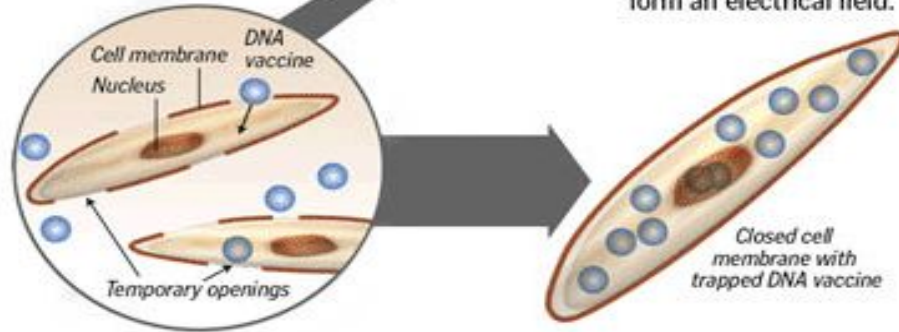
How electroporation delivers DNA vaccines



- 1 Syringe and needle electrodes are inserted into selected muscle tissue, and the DNA vaccine is injected.



- 2 Controlled, millisecond electrical pulses are applied to the needle electrodes, which then form an electrical field.



- 3 The electrical field creates temporary openings in the cell membrane, allowing significantly greater amounts of the DNA vaccine to enter cells.
- 4 The trapped DNA enables cells to produce antigen designed to control cancer and chronic infectious diseases such as HIV. The antigen can also trigger antibody production to prevent diseases.

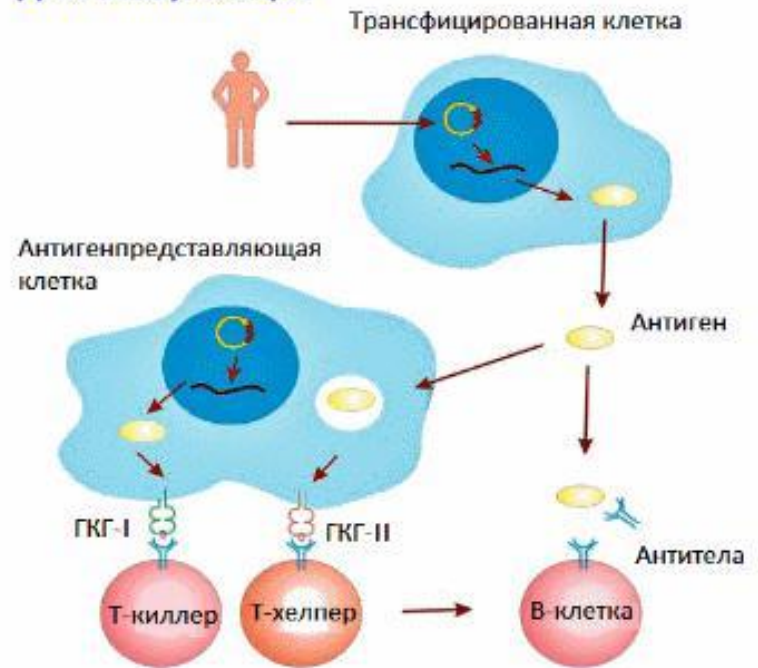
It was later revealed that the cloned cDNA can be also introduced into cells by intramuscular injection of a solution with a lots of plasmid carrying the corresponding DNA. To do this it is necessary to have DNA in 10^3 - 10^4 times more than in bombardment of the microparticles. **This method, called the Genetic immunization can be used to immunize animals.**

Plasmid DNA represents circular molecule covalently closed length of 4.6 base pairs. It has a site responsible for initiating transcription (promoter), gene of protective protein, gene that provides the resistance of cells to antibiotics (ampicillin), and the site of replication of plasmid DNA. **Plasmid DNA replication occurs only in bacterial cells, whereas transcription of the gene of protective protein is carried out only in mammalian cells.**

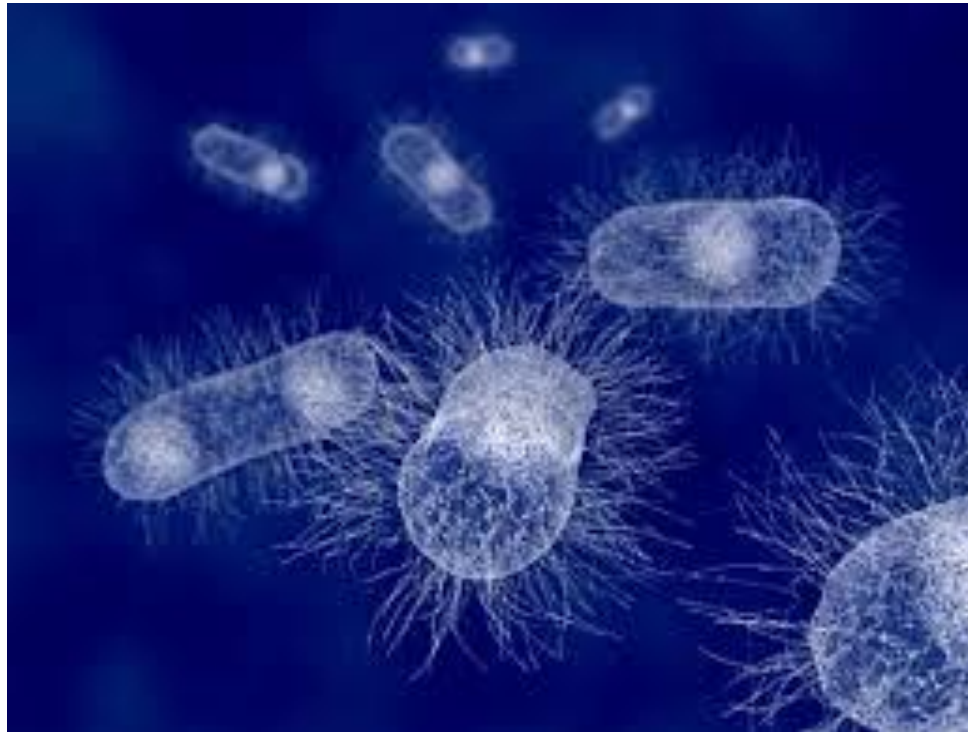
МЕТОДЫ ПОЛУЧЕНИЯ ДНК-ВАКЦИН:

- Чужеродные белки, производящиеся клетками организма-хозяина, процессируются протеосомами и представляются клетками иммунной системы, вызывают специфический иммунный ответ
- Синтезируемые чужеродные белки могут также высвобождаться во внеклеточное пространство. Такие антигены вызывают в основном развитие гуморального иммунного ответа
- ДНК-вакцины сохраняются в организме 3-4 недели. За это время они успевают индуцировать Т- и В-клеточный иммунитет

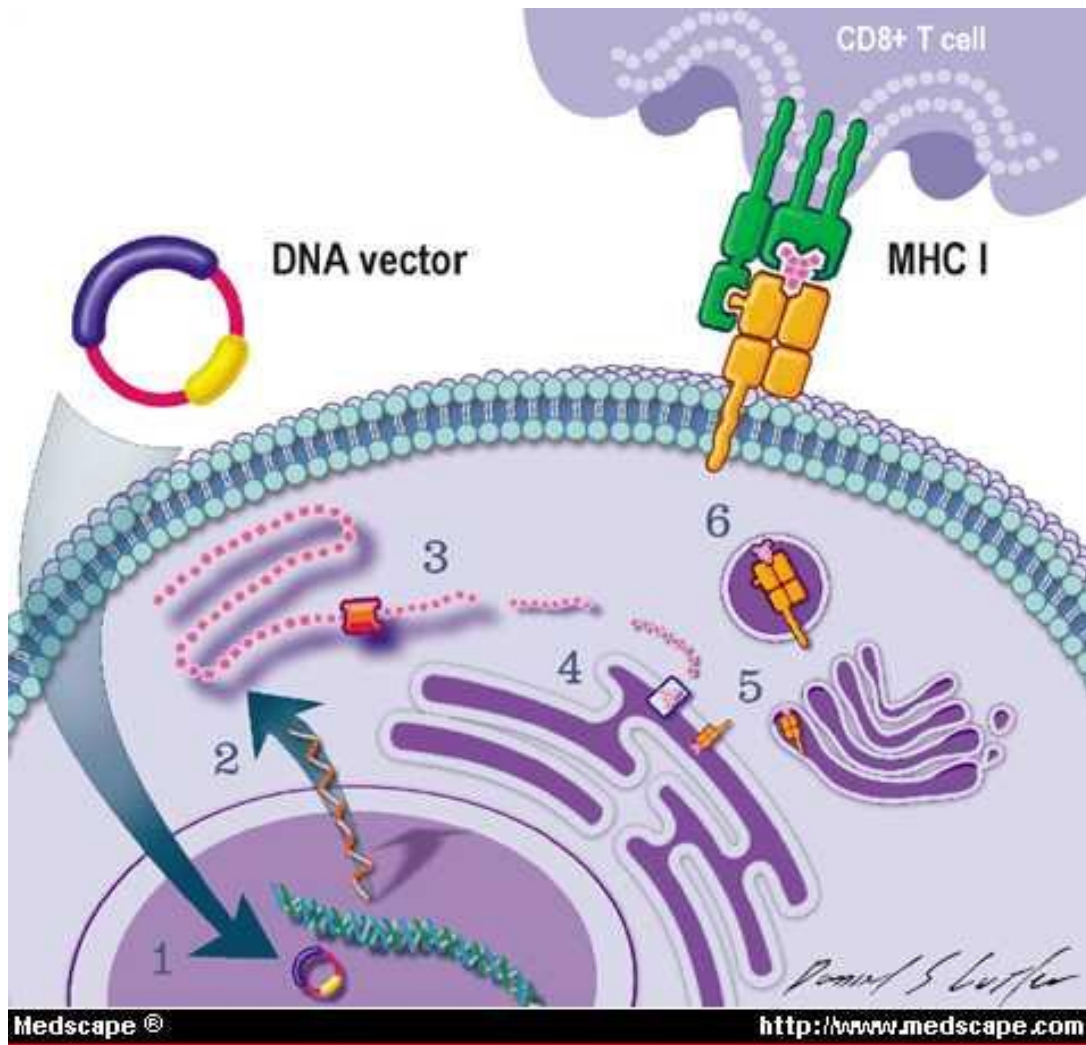
ДНК-иммунизация



***Shigella flexneri* was created to facilitate the delivery of DNA into animal cells during genetic immunization.**

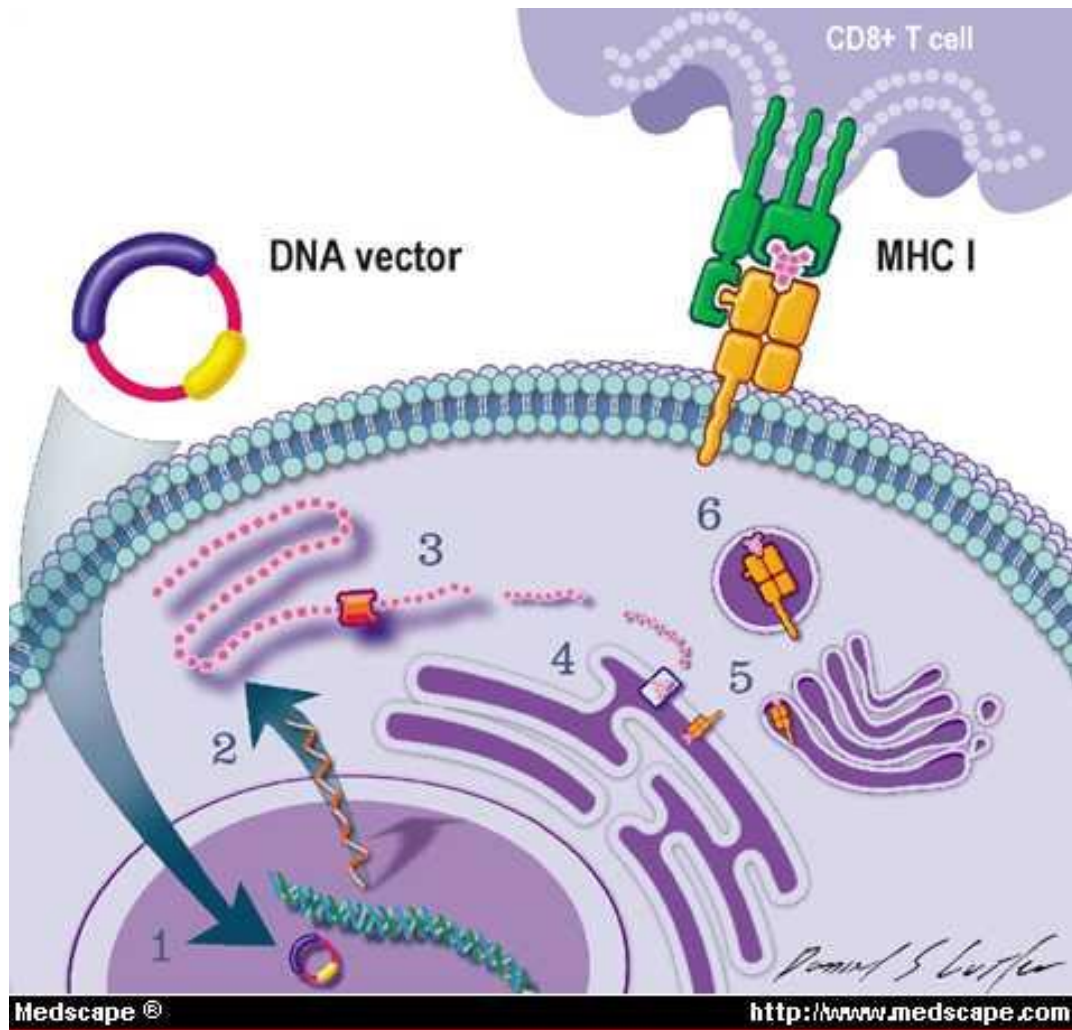


Modified strain of *Shigella flexneri* was created to facilitate the delivery of DNA into animal cells during genetic immunization. This bacterium enters the epithelial cells of animals by phagocytosis, and plasmid DNA which is presented in it enters the host cell cytoplasm, where transcription and translation of gene under the control of a eukaryotic promoter is occurred. *Shigella* is pathogen, that is why it can not be used to deliver DNA. Its non-pathogenic strain can be obtained by entering a deletion in the gene of *asd*, encoding the enzyme aspartate-beta-semi-aldehyde dehydrogenase, which is involved in the synthesis of cell wall components namely diaminopimelic acid. Strains with mutations in the gene *asd* grow only in the presence of diaminopimelic acid and can be used to deliver plasmid DNA in the epithelial cells of animals, as they are not proliferating.



At present current trends in the development of recombinant vaccines is the construction of various **DNA vaccines based on a single plasmid vector**. It should be noted that DNA vaccines have the safety of inactivated vaccines and efficacy of alive ones. Protective protein genes of several pathogens and cytokine genes - regulators of the immune response can be integrated in one plasmid DNA. Experimental studies of DNA vaccine were made from human immunodeficiency virus, influenza, rabies, hepatitis B and C, herpes simplex, warts, and tuberculosis pathogens and parasitic diseases (malaria and leishmaniasis).

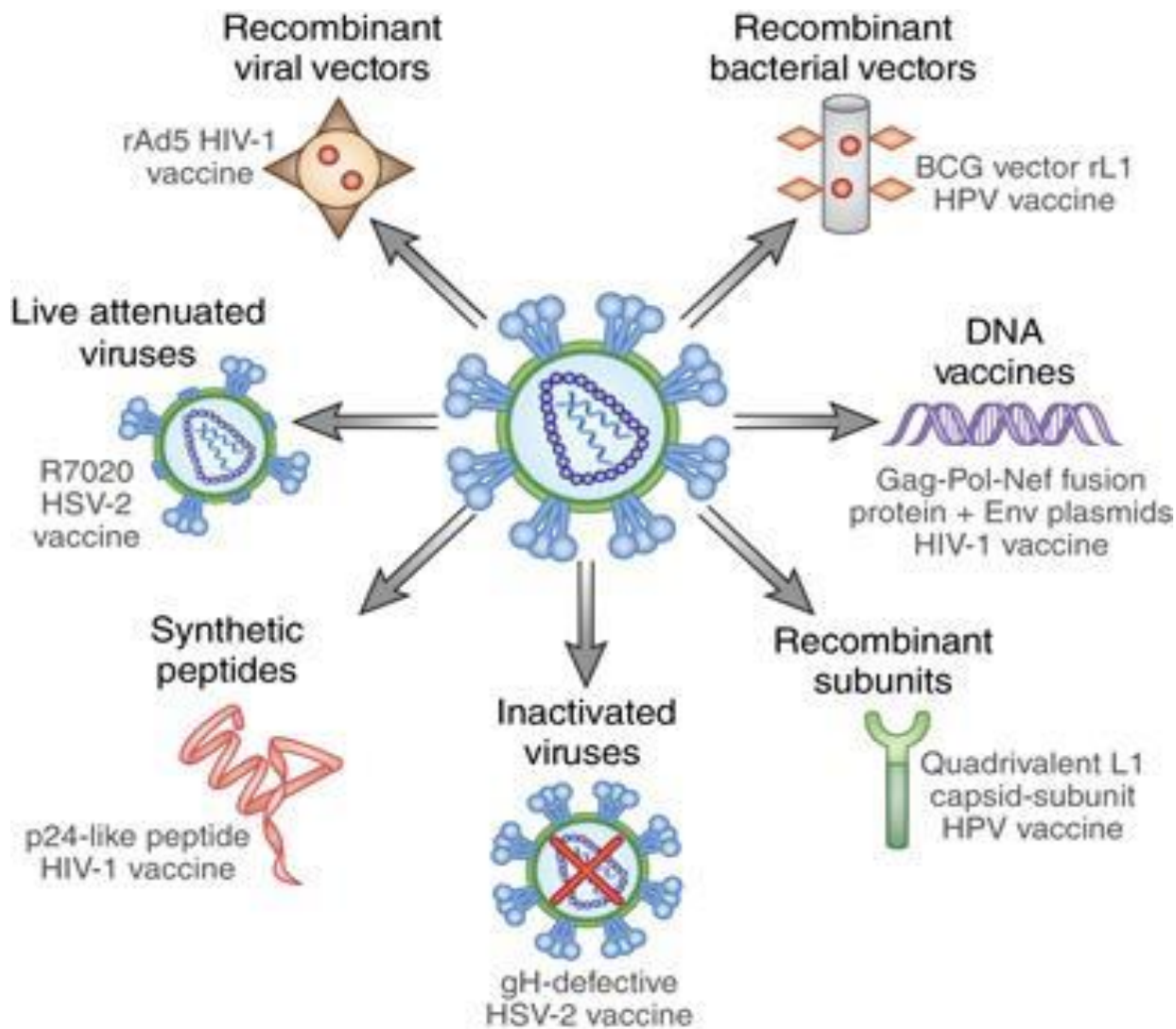
Effectiveness of immunization of DNA vaccines is obvious, but it will take a lot of effort for the practical implementation of a new approach to prevention of infectious diseases of animals. However, safety issues of vaccines from plasmid DNA for human remains unsolved. The risk of mutagenic effects and immunopathological reactions in response to the DNA vaccine is also not defined. There is no clear idea of the side effects of the resulting antigens and immune response mediators.



Plasmid DNA is absorbed by the cells of animals in a small amount (0.01-1.0%), and most of it is quickly destroyed. DNA penetrated into the cell is transported to the nucleus of the cell and transcribed by *DNA-dependent RNA polymerase 2* with the formation of *messenger RNA*, which in the cytoplasm provides a synthesis of protective protein. Plasmid DNA functions in cells for a long time (up to 3-6 months.). In the body of the animal, plasmid DNA does not replicate and embedded in the chromosomes, and does not formed antibodies. Protective protein synthesized in the cells is cleaved in the cytoplasmic proteasome into short peptides (8-10 amino acids). **The last associated with the molecules of Major Histocompatibility complex (MHC) class 1 and are transported to the cell surface.** Synthesized protective proteins can be transported out of the cell into the extracellular space in the free unsplit state. It binds to the antigen-presenting cells (macrophages), penetrates in it by endocytosis and are cleaved in endosomes into short fragments (10-20 amino acids).

Fragments of proteins are combined with the molecules *MHC class 2* and integrated into the surface membrane of the cell. On the cell surface *antigen + MHC 2* complex is recognized by *T-helper* cells. *B* cells are transformed to antibody producing cells under the influence of protective protein and antigen-activated *T-helper* cells.

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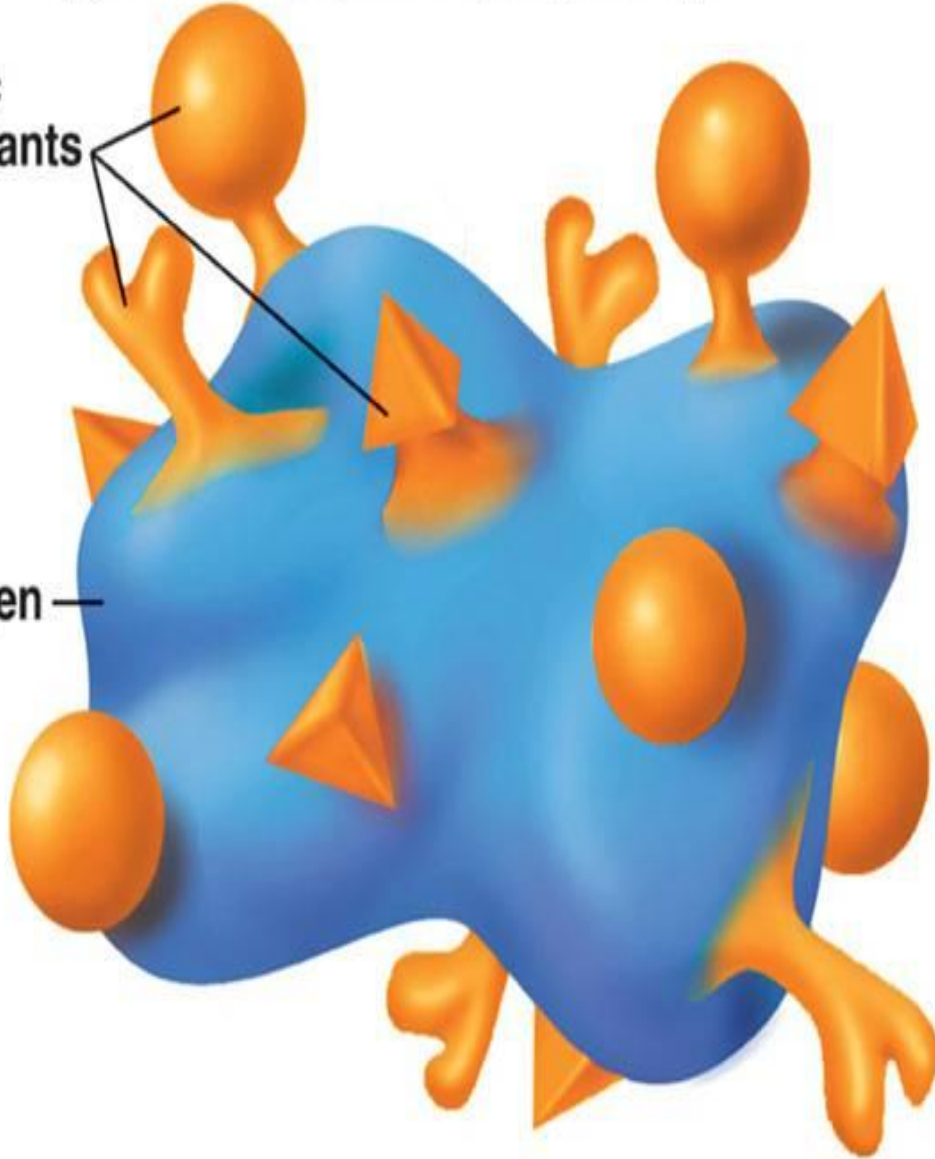
Synthetic peptide vaccines.

The idea of using synthetic peptides as vaccines was born when studying cellular and molecular mechanisms of immunity. Nowadays polysaccharides analogous to natural-antigens, for example, Salmonella polysaccharides are synthesized and tested. Producing vaccines by Recombinant DNA technology opened new perspectives in the development of synthetic vaccines. Production of the latter can replace the existing bacterial and viral vaccines with extraneous antigenic determinants, proteins and other substances that cause side effects.

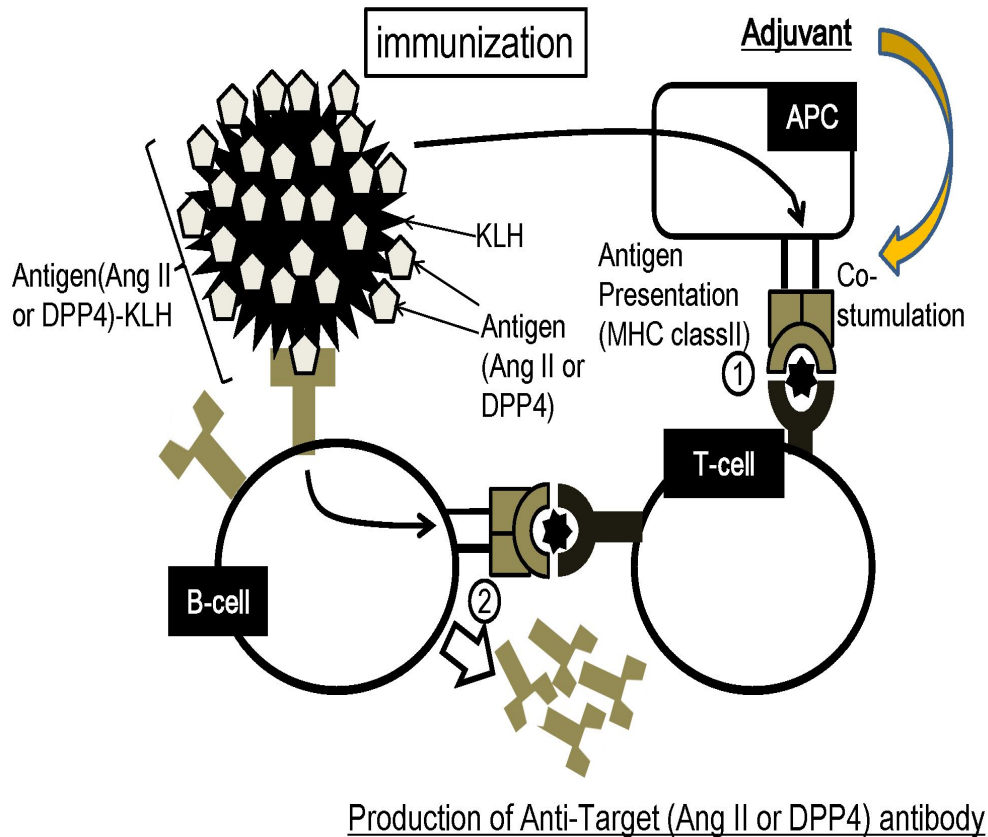
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Different
antigenic
determinants

Antigen



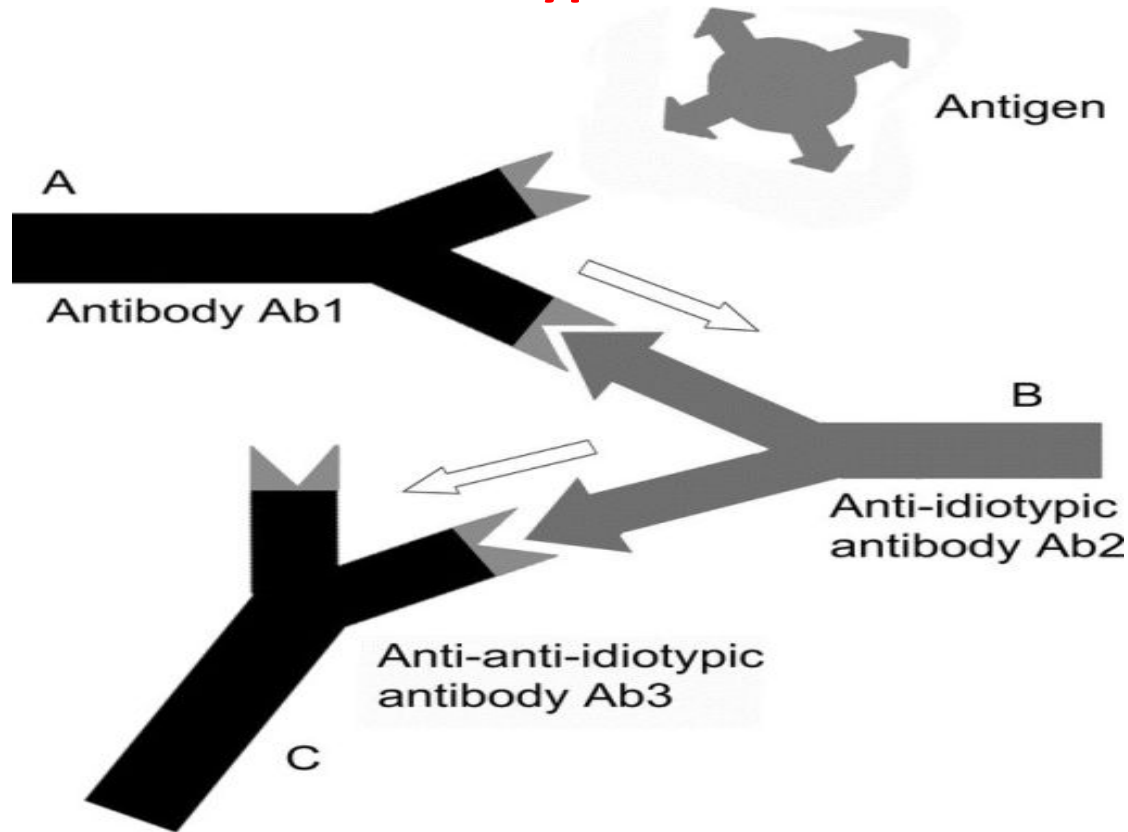
Synthetic peptide vaccines. Then the next question is: can a small part of the protein molecule (domain) serve as an effective subunit vaccine and induce the production of antibodies? Intuitively, it seems that those domains that are available for the antibodies (ie, those that are on the surface of the virus), have immunogenic properties, and internal domains are negligible, unless they affect the conformation of the immunogenic domain. If this assumption is true, the short peptides that mimic epitopes (antigenic determinants) can be used to create vaccines. The idea of using synthetic peptides as vaccines was born when studying cellular and molecular mechanisms of immunity. Nowadays polysaccharides analogous to natural-antigens, for example, *Salmonella* polysaccharides are synthesized and tested. Producing vaccines by Recombinant DNA technology opened new perspectives in the development of synthetic vaccines. Production of the latter can replace the existing bacterial and viral vaccines with extraneous antigenic determinants, proteins and other substances that cause side effects.



At Scripps Clinic Research Institute and at the Institute of Virology of Animals (USA) polypeptides corresponding to several areas of the protein **VP1 of FMDV** were synthesized. In further studies, they found that one of the polypeptides including the area from **141 th to 160 th amino acid of VP1** by injection with adjuvant and in combination with keyhole hemocyanin (KLH) causes **antibody synthesis in guinea pigs to the virus and rabbits.**

Formation of immunity succeeded by injection of synthetic peptide *Streptococcus pyogenes M* protein just 20 amino acids in length. Such immunogenic oligopeptides can be the basis of safe vaccines against streptococcal infections that cause rheumatic fever and related heart disease.

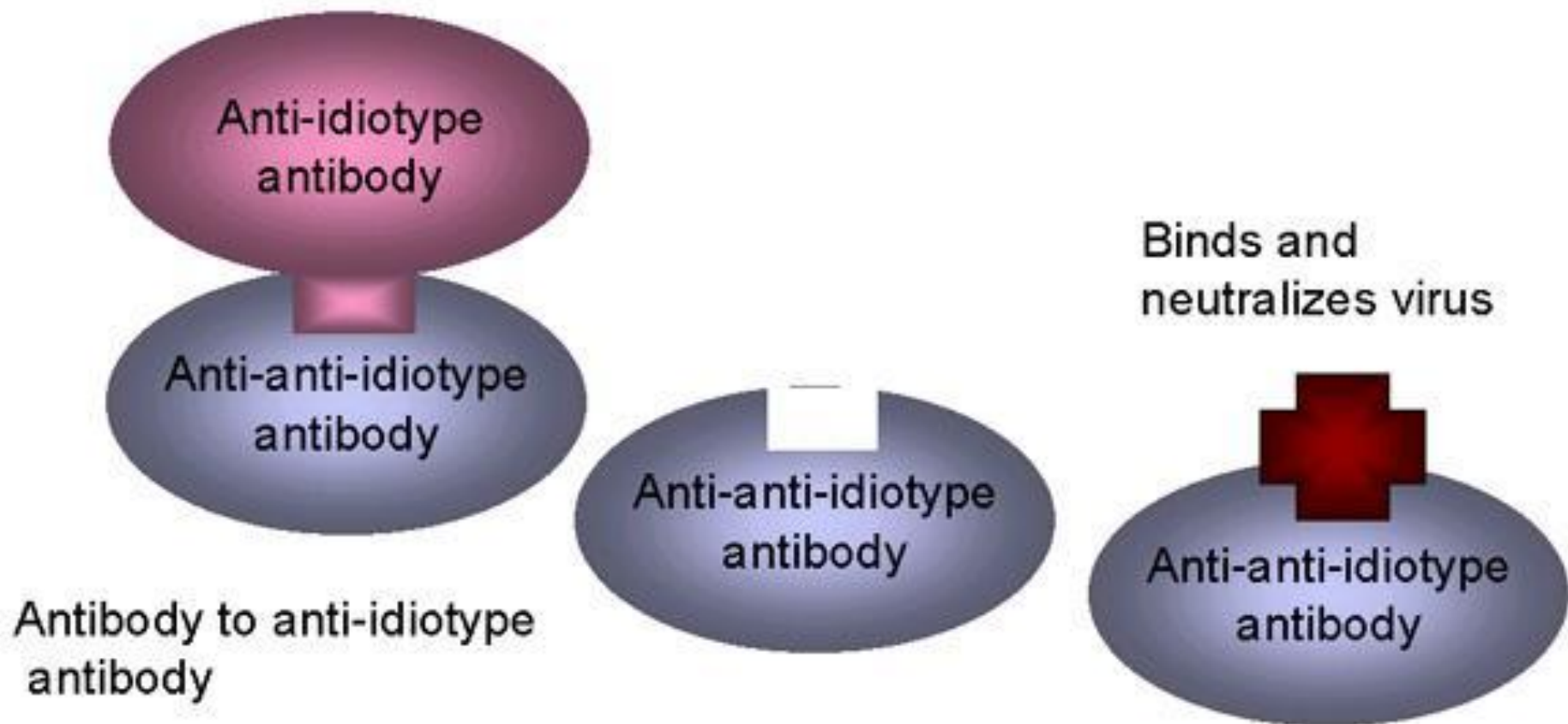
Anti-idiotypic vaccines



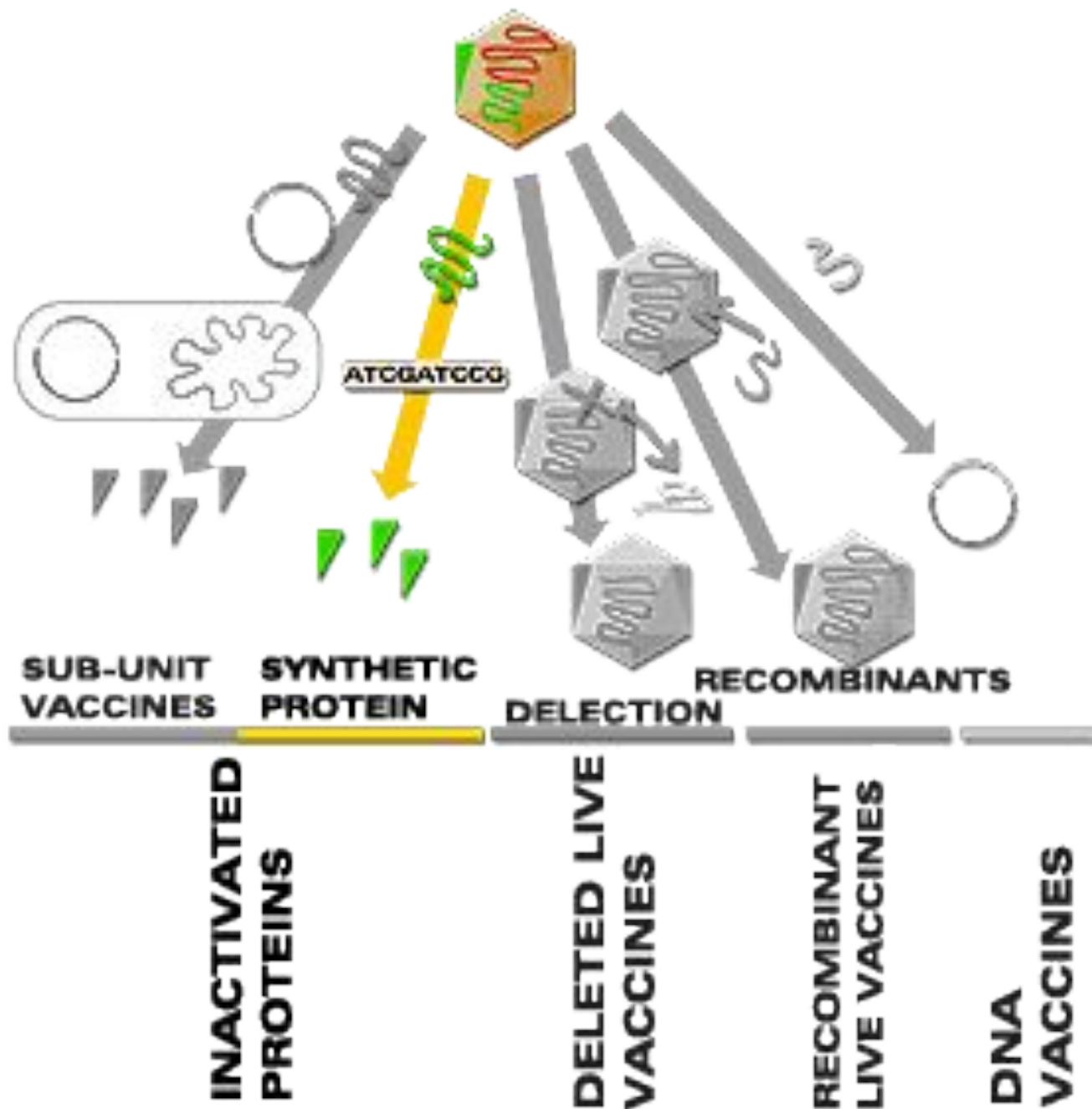
Idiotypic vaccines have several advantages over traditional prophylactic preparations. First of all, immunoglobulin nature of **AIAT prevents reversion of live attenuated microorganism in virulent form**. Furthermore, as it is known, synthetic peptides corresponding to parts of the primary amino acid sequence, created by chemical synthesis or molecular cloning is not always able **to maintain a native three-dimensional structure necessary for the induction of antibodies desired specificity** and immunogenicity, which was confirmed on the model plague pathogen in contrast to AIAT, selected precisely for conformational specificity as "mirrors" of the antigenic epitope.

Anti-idiotypic antibody cont 2

Use anti-idiotypic antibody as injectable vaccine

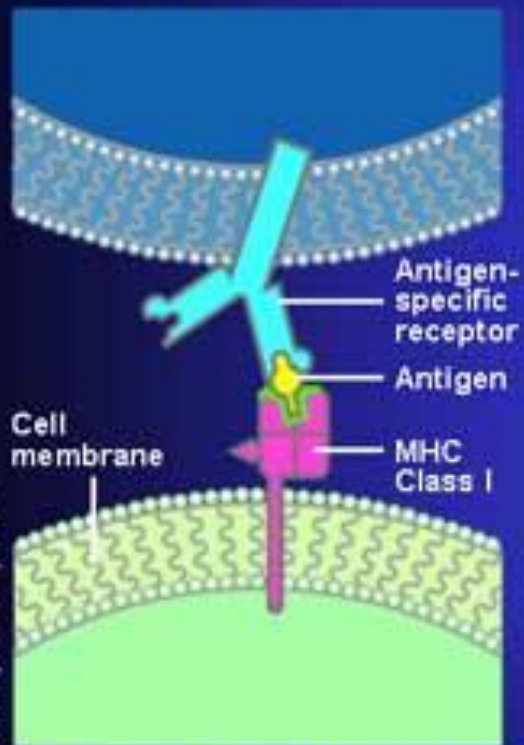


NEW GENERATION VACCINES



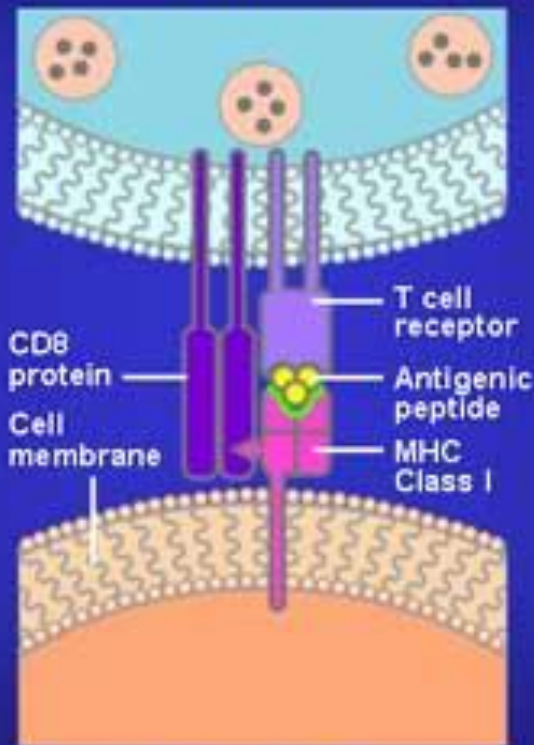
Antigen Receptors

B cell



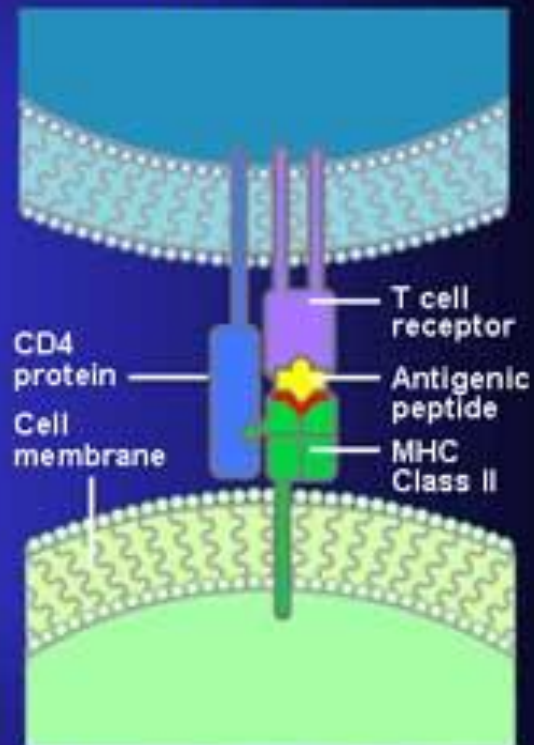
Antigen-presenting cell

Killer cell



Infected cell

Helper T cell



Antigen-presenting cell

Adapted by Joanne Kelly, ©2004.

Edible vaccines

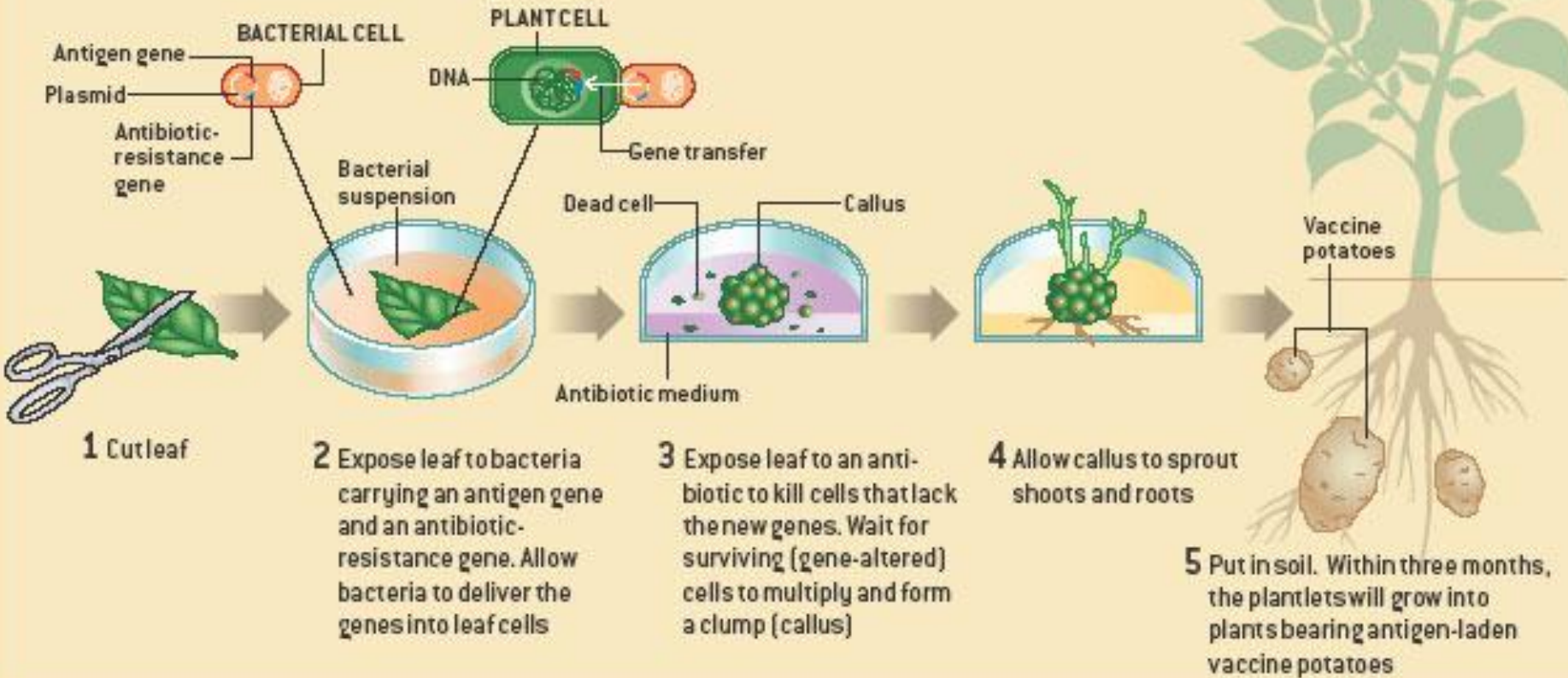
Advances in genetic engineering have opened up new opportunities for the production of recombinant proteins. For this purpose bacterial cells, yeast, mammals and insects are widely used. However, they have several drawbacks. **In the cells of prokaryotes posttranslational modification and correct folding of polypeptide chains of many eukaryotic proteins does not occur.** Mammalian and insect cells are deprived of such shortcomings, but the use is limited by high production costs of recombinant proteins yield.

In comparison to aforementioned plant expression systems have a number of features and benefits. First of all, it should be noted that in higher plants **glycosylation and protein folding occurs, similar to those in mammalian cells.** Cultivation of plants does not require expensive equipment, in contrast to animals, plant cells **do not contain viruses and prions pathogenic for human** and thus serve as a safe source of recombinant proteins. In addition, **transfer of exogenous DNA fragments into the plant genome and the regeneration of plants is much easier as compared to animals.** Revolutionary trends in modern vaccinology is the development of vaccines based on transgenic plants in genome of which corresponding fragment of pathogenic microorganism's genome is inserted. Transgenic plants-producers of epitopes of disease agents are called "edible vaccines".

HOW TO MAKE AN EDIBLE VACCINE

One way of generating edible vaccines relies on the bacterium *Agrobacterium tumefaciens* to deliver into plant cells the genetic blueprints for viral or

bacterial "antigens"—proteins that elicit a targeted immune response in the recipient. The diagram illustrates the production of vaccine potatoes.



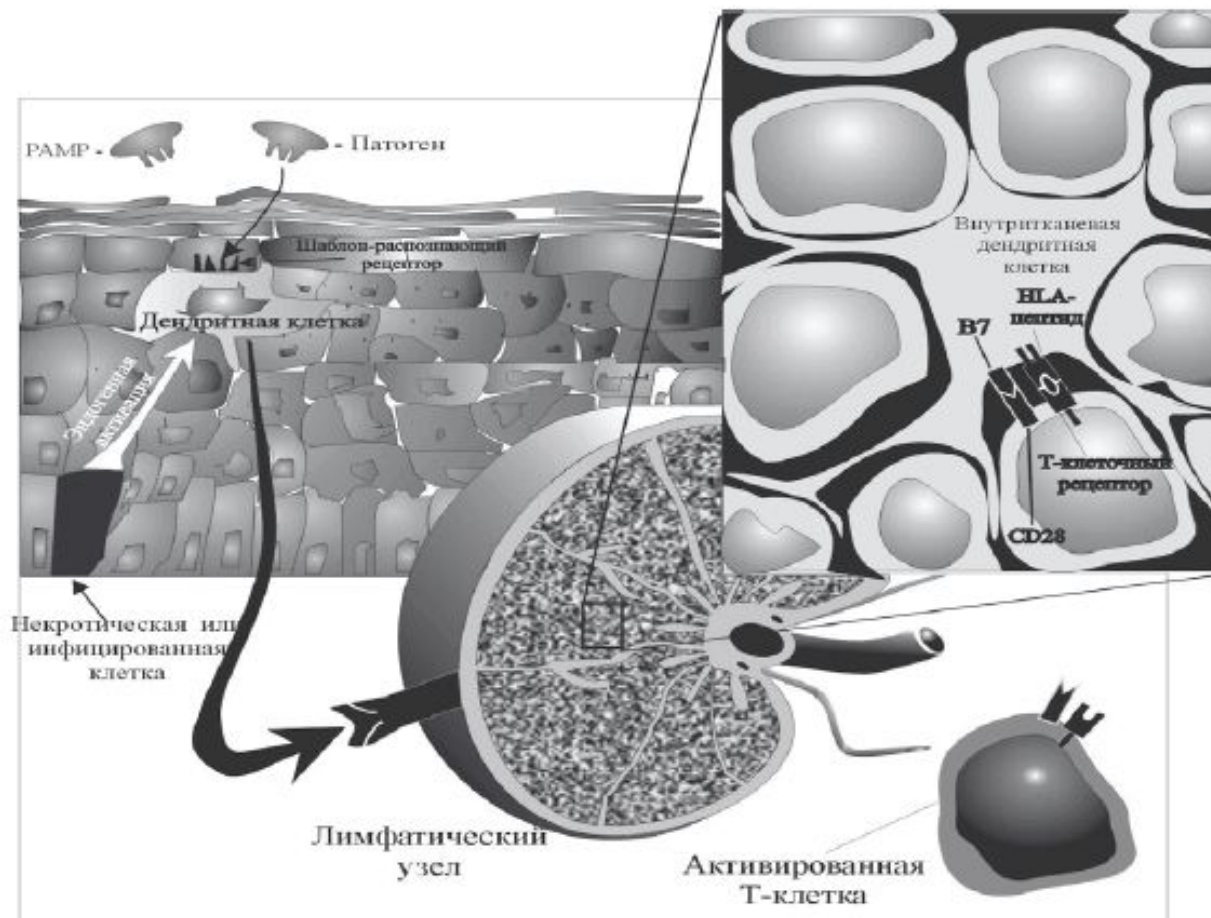


Рисунок 4. Роль дендритных клеток в иммунном ответе.

На рисунке показано, как инфицированные или повреждённые патогеном клетки ткани активируют дендритные клетки барьерных органов (клетки Лангерганса). Последние мигрируют в зону повреждения, где распознают патоген по его молекулярным шаблонам. Захватив определённую порцию патогена, клетки Лангерганса покидают очаг и мигрируют в регионарный лимфатический узел. На пути миграции они называются вуалевидными клетками. В лимфатическом узле прибывшие дендритные клетки презентуют антиген специфическим, но «наивным» (некомпетентным) Т-хелперам. Благодаря антигенной презентации, наивные Т-клетки активируются и приобретают компетентность, способствуя инициации иммунных реакций.

The mechanism of immunization by Edible Vaccines is based on antigen-presenting ability of peritoneal macrophages of the small intestine of mammals.

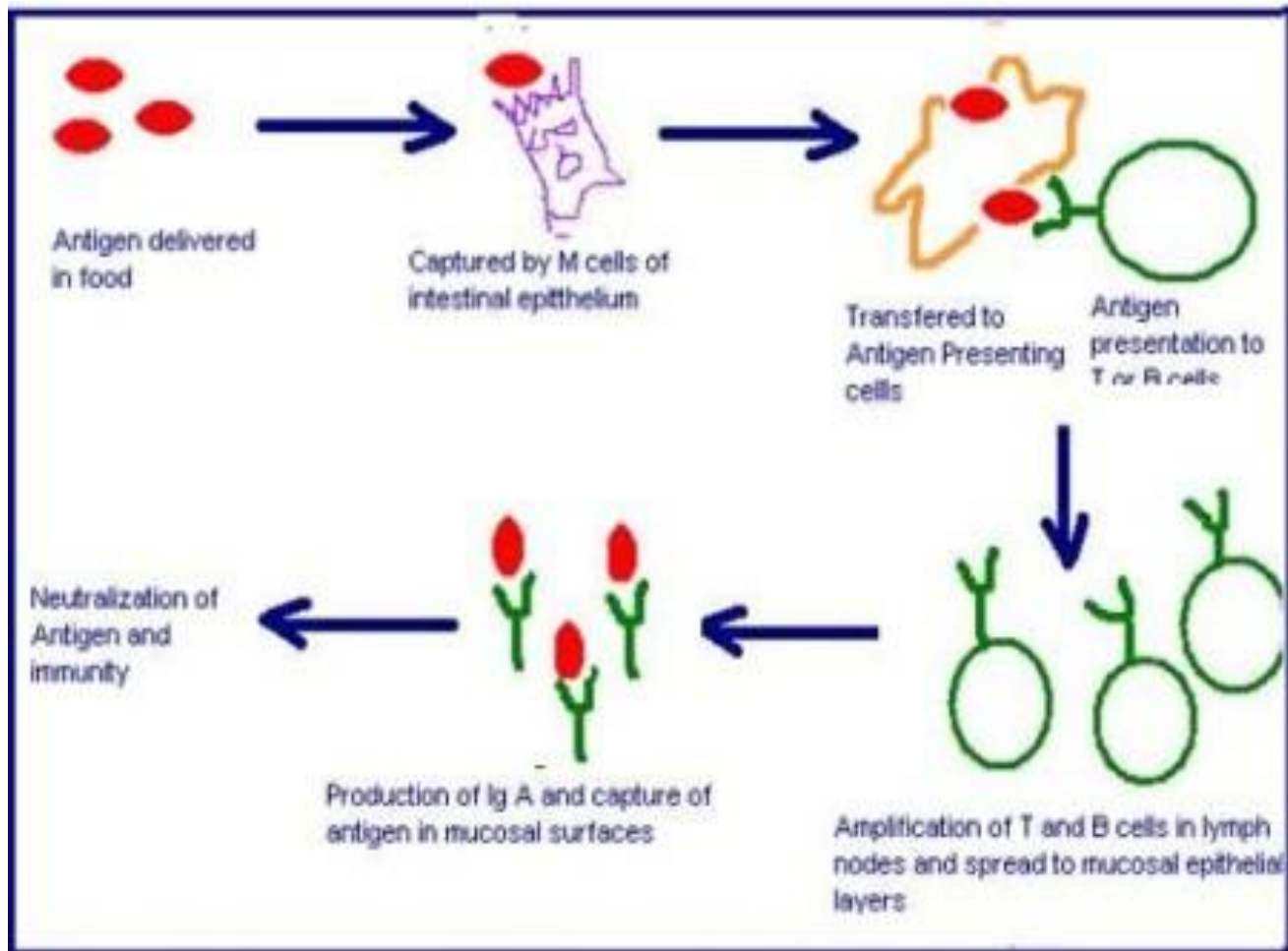
Secretory **immunoglobulin IgA** transported to the surface of the mucous membrane, where they bind to the foreign agent and prevent their penetration into the body.

The first such vaccine was received in 1992: a transgenic tobacco plant became producing "Australian" antigen. Derived from plants and partially purified antigen injected into mice caused a powerful immune response similar **to hepatitis B vaccine**.

In 1998, using potatoes, producing B-subunit of **cholera toxoid** severe protection of mice from cholera was obtained. In the same year, 10 of the 11 volunteers who received 100 g of raw potato, producing antigens of **enterotoxigenic *Escherichia coli***, began to develop antibodies to this pathogen in intestinal mucosal.

«Potato» vaccine to the pathogen of **diarrhea and hepatitis B** is experiencing nowadays with promising results. Vaccines against **rabies, foot and mouth disease** are tested on animals. The research is conducted on the basis of transgenic potato, lettuce, corn, spinach, alfalfa, etc. Today, transgenic plants-producers of different types of antibodies to several epitopes of antigens (**staphylococcus, streptococcus, herpes simplex virus, cancer embryonic antigen**) are received.

Transgenic plants are considered as a potential source of low-cost human immunoglobulins and animals.



Вакцина



это медицинский препарат, который изготавливается из ослабленных или убитых микроорганизмов.

Прививка- это процедура введения вакцины

Сыворотка



это медицинский препарат, который содержит готовые антитела.

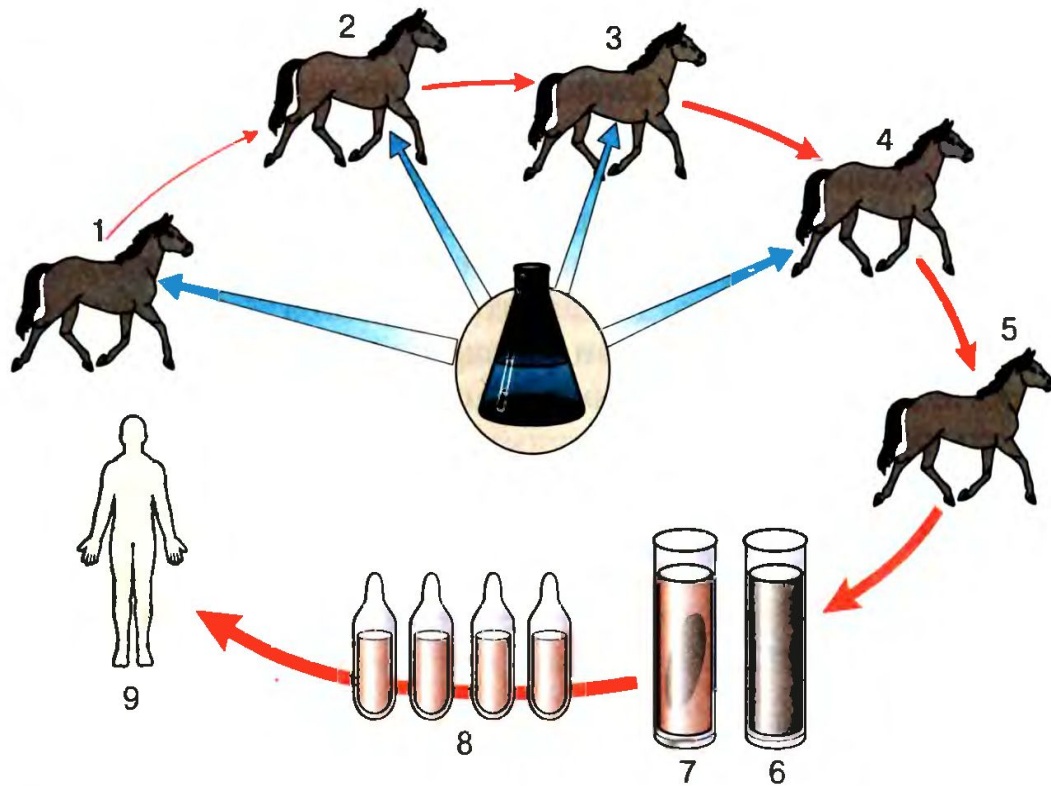


Рис. 48. Изготовление антидифтерийной сыворотки: 1, 2, 3, 4 — многократная вакцинация лошади (ей вводят дифтерийный яд — токсин), в ее организме вырабатываются антитела против дифтерийного яда — антитоксины; 5 — взятие крови с антитоксинами, уничтожающими дифтерийный яд; 6, 7 — приготовление сыворотки крови, содержащей антитоксины (освобождение от форменных элементов, получение плазмы крови, удаление фибриногена — получение сыворотки крови, содержащей антитоксины, выработанные в организме лошади); 8 — ампулы с антидифтерийной сывороткой; 9 — введение сыворотки здоровому человеку для профилактики заболевания или больному — для излечения