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DERLEME
REVIEW

Next Generation Vaccines

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Abstract

For centuries, mankind has been aware that prevention is more valuable than cure and has sought appropriate ways to do so. The adventure of vaccination, known as the most effective protection method, began with studies against smallpox. It continued when Edward Jenner administered the vaccinia virus to a child in 1796, which he received from a woman infected by a cow. Louis Pasteur observed that the virus administered in 1798 eliminated the smallpox virus after a few months, so the smallpox vaccine was first discovered and applied. The concept of inactivated vaccines emerged during the collaboration between Robert Koch and Louis Pasteur. Inactivated vaccines against plague, cholera, and typhoid emerged around the end of the nineteenth century. In 1948, the first combined vaccine against diphtheria, tetanus and pertussis was produced. After the second half of the 20th century, new applications began to be introduced. Then, cell culture studies for viral vaccines started. The effect of advancing technology began to be felt in vaccines over time and new generation vaccine studies started. With cloning, the foundation of recombinant vaccines and thus new-generation vaccines was laid. Scientists focused on next-generation vaccine studies and introduced vaccines such as viral vector-based vaccines, RNA-based vaccines, Subunit vaccines, Virus-like particle vaccines and Marker vaccines into vaccine technology.

Anahtar Kelimeler: New generation vaccines, recombinant protein, vaccine types

Yeni Nesil Aşılar

Öz

İnsanlık yüzyıllardır önlemenin tedavi etmekten daha iyi olduğunu biliyor ve bunu başarmanın yollarını arıyor. En etkili korunma yöntemi olarak kabul edilen aşılama serüveni çiçek hastalığına ilişkin çalışmalarla başladı. Bu durum, 1796'da Edward Jenner'ın, bir inekten enfekte olan bir kadından aldığı aşı virüsünü bir çocuğa vermesiyle devam etti. Louis Pasteur 1798 yılında uygulanan virüsün çiçek virüsünü birkaç ay sonra ortadan kaldırdığını gözlemlemiş ve böylece ilk kez çiçek aşısı bulunup kullanılmıştır. Robert Koch ve Louis Pasteur tarafından sürdürülen bu süreç, inaktive aşı kavramının ortaya çıkmasına yol açtı. 19. yüzyılın sonlarına doğru veba, kolera ve tifoya karşı inaktif aşılar geliştirildi. 1948 yılında difteri, tetanoz ve boğmacaya karşı ilk kombine aşı üretildi. 20. yüzyılın ikinci yarısından sonra yeni uygulamalar ortaya çıktı. Viral aşılar için hücre kültürü çalışmaları başladı. Zamanla teknolojik gelişmelerin aşılar üzerindeki etkisi hissedildi ve yeni nesil aşılar üzerinde çalışmalar başladı. Klonlama, rekombinant aşıların ve yeni nesil aşıların temelini attı. Bilim insanları yeni nesil aşı çalışmalarına odaklanarak viral vektör bazlı aşılar, RNA bazlı aşılar, alt birim aşılar, virüs benzeri parçacıklı aşılar ve marker aşılar gibi aşıları aşı teknolojisine kazandırdı.

Key Words: Aşı çeşitleri, rekombinant protein, yeni nesil aşılar

INTRODUCTION

The process of bringing and applying the agent or agents that will cause infection to the formulation to be given to the organism by various methods and creating immunization against those agents after the application is called "vaccination" and the biological substances used for this process are called "vaccine" (1). The first vaccination history back almost 4 centuries (2). While people did not even know the definition of microorganisms, they struggled for immunization. After being infected, the impossibility of treatment together with the difficulties of the period and the occurrence of deaths even from a simple infection forced people to find ways of protection against diseases. In this context, they first tried to provide immunity against this disease by drying the crusts of the wounds of people infected with smallpox, scratching the skin of healthy people and applying them to them (3). Today, taking preventive measures against the disease rather than treating it is the priority in the fight against infections.

Vaccinology, known as vaccine science, is a multidisciplinary science. Fields such as immunology, microbiology, molecular biology, biochemistry, and statistics are closely related to this science. The first goal of vaccination is to protect against infections. However, with the recently developing science, it is also used in cases such as cancer vaccines, birth control or autoimmune diseases, allergies, such as reducing the immune response by combining new generation technologies (4). When vaccines were first administered, they were made using purified attenuated live viruses or inactivated microorganisms. Later on, more refined methods were used. Applications such as the creation of toxoid from a protein toxin and its use in treatment, the creation of purified and inactivated virus, the development and use of virus-like particles and purified polysaccharides have started to take place in science. Vaccines are usually made in a type that includes all microorganisms, purified macromolecules, combined antigens, recombinant vectors with later developing technology, synthetic peptides, or nucleic acids such as DNA RNA. With these developing vaccine types, production processes have become more technological (5).

Next Generation Vaccines

Pathogens become better understood as molecular biology and microbiological tools progress. Wolf et al. (6) discovered that mice injected with a plasmid containing a cloned protein also expressed a cloned transgenic protein in the plasmid DNA. These observations prompted the development of a new immunization approach, ushering in the age of next-generation vaccines. The first tactic employed for these novel vaccines was the DNA-based technology, followed later by the invention of viral vectors for immunization, such as adeno-associated virus (AAV), lentiviral or adenoviral vectors, and more recently RNA-based vaccines (7). With the intensive expansion of genome-based studies, different methods have started to be developed in vaccines. The advantages offered by technologies that enable the understanding of the entire genome of the microorganism have created a perspective for vaccine research. Sequencing is of great importance in determining the pathogenic profiles of similar or different types of bacteria (8). The whole genome sequence is sequenced with bioinformatics tools and the dominant pathogenic strain is identified in the field. With these genomic analyses, new-generation vaccines or antimicrobial molecules are designed against pathogenic bacteria. Genome sequences enable the identification of molecules with vaccine potential, regardless of whether the agent is produced in vivo or in vitro. In silico analysis of the genome sequence is the starting point for vaccine design. This innovative approach, which is different from conventional vaccination science, is called "Reverse vaccinology" (9). Although bioinformatics information on new potential candidate vaccines is available, in silico analysis must also be performed. When genomic data is integrated with advanced techniques like as in vivo expression technology (IVET), signature-tagged mutagenesis (STM), DNA microarrays, and proteomics, new surface antigens or virulence factors can be experimentally identifiedAll of these studies, known as "functional genomics," equip us with tremendous tools for studying the genome (10).

As a result, these innovative vaccines contain only a specific viral/bacterial antigen rather than utilizing the entire pathogen, resulting in an improved safety profile. However, developing such vaccines necessitates a more in depth understanding of viral/bacterial structures, as well as the interaction between viral/bacterial proteins and host cell receptors. Next generation vaccinations require a protracted preliminary study period before they may be developed. A reverse vaccinology strategy was used to develop a vaccine against the human pathogen Neisseria meningitidis serogroup B. A recombinant vaccine against Hepatitis B (HBV) was prepared using the subunit vaccination method, which is based on a specific immunogenic antigen, and a vaccine against whooping cough was prepared by highly purifying 3 proteins of Bordetella pertussis (11).

Recombinant Protein Vaccines

Recombinant protein vaccines use recombinant viral or bacterial structural proteins to boost the immune system. Because the immune system's humoral and cellular elements recognize and respond immunologically to specific pathogen locations (either toxins isolated from the organism or surface antigens isolated from the organism, etc.) this has led to the development of vaccinations based on pathogen components i.e. protein components that have a protective function (12). The basic strategy in recombinant vaccine technology is to clone one/several genes from different etiological agents and transfer them to bacterial, yeast, mammalian, and insect cells are capable of replicating the antigenic determinant's DNA. The important point here is that several considerations should be considered before selecting the system for antigen expression. The main features that determine the efficiency of producing the efficacy of the vaccine is influenced by various factors including the expression level of the antigen gene within the specified vector and promoter, the inclusion of a selection marker, and whether post-translational modifications are facilitated by the recombinant vector (Figure 1). The most common expression systems are bacterial extensively utilized systems because of their case of use and high-level expression capabilities. Despite the developing technology in the field of vaccination, it is still difficult to develop vaccines for persistent infections

such as HIV and mycobacteria. In such cases, the immunogenic part of the pathogen is produced as a recombinant protein and an immune response is created in the organism. The bulk of vaccines being researched now are made up of highly purified recombinant proteins or pathogen components (13). This technology enables the development of immunogenic protein-based vaccines for agents that are difficult to produce by culture. Following the identification and production of recombinant antigens of malaria and SM28 protein of schistosomiasis, which is one of the research within this scope, it enables the development of appropriate vaccine formulation by proceeding to clinical trials (14). The Hepatitis B (HBV) vaccine is one of the recombinant vaccines that has been confirmed to be effective and is currently licensed for human use. It contains the recombinant Hepatitis B viral surface antigen (HBsAg) is created by DNA transfected yeast or mammalian cells (15). Although vaccines based on recombinant proteins provide significant advantages over conventional vaccines in terms of safety and production cost; yet, most of them demonstrate limited immunogenic effects when administered alone, so effective and appropriate adjuvants should be used to create a strong and long-lasting immunological response (16). It is much more difficult to stimulate a cellular immunological response against intracellular pathogens using conventional vaccination techniques. Live attenuated pathogen vaccinations are can elicit such a response, can cause possible risks that cannot be ignored, such as increased virulence and pathogenicity in vulnerable hosts, although not frequently. Recombinant vaccines, on the other hand, are based on the expression of one or more defined antigens by plasmids or apathogenic bacterial/viral vectors to stimulate immunity against the pathogen and are administered with adjuvants (17). When considering recombinant protein vaccines, e.g. diphtheria or tetanus toxoid vaccines, vaccines based on purified macromolecules allow protection from the major risks mentioned, for example, undesired pollutants may be co-purified, and toxoids may be converted into dangerous forms. This approach also addresses the issue of a lack of purified antigenic components in enough quantities (18). Some recombinant proteins have low immunogenicity and aluminum salt, currently the only immunological adjuvant licensed for human use, is sometimes insufficient. Therefore, to improve the efficacy of such vaccines, molecular biology methods can be used to characterize more effective adjuvants. Early studies on the design of adjuvants mostly focused on the use of cytokines and especially one of them, interferon g (IFN-g). IFN-g, one of the most studied cytokine adjuvants, induces an immune response even when given alone to the organism.

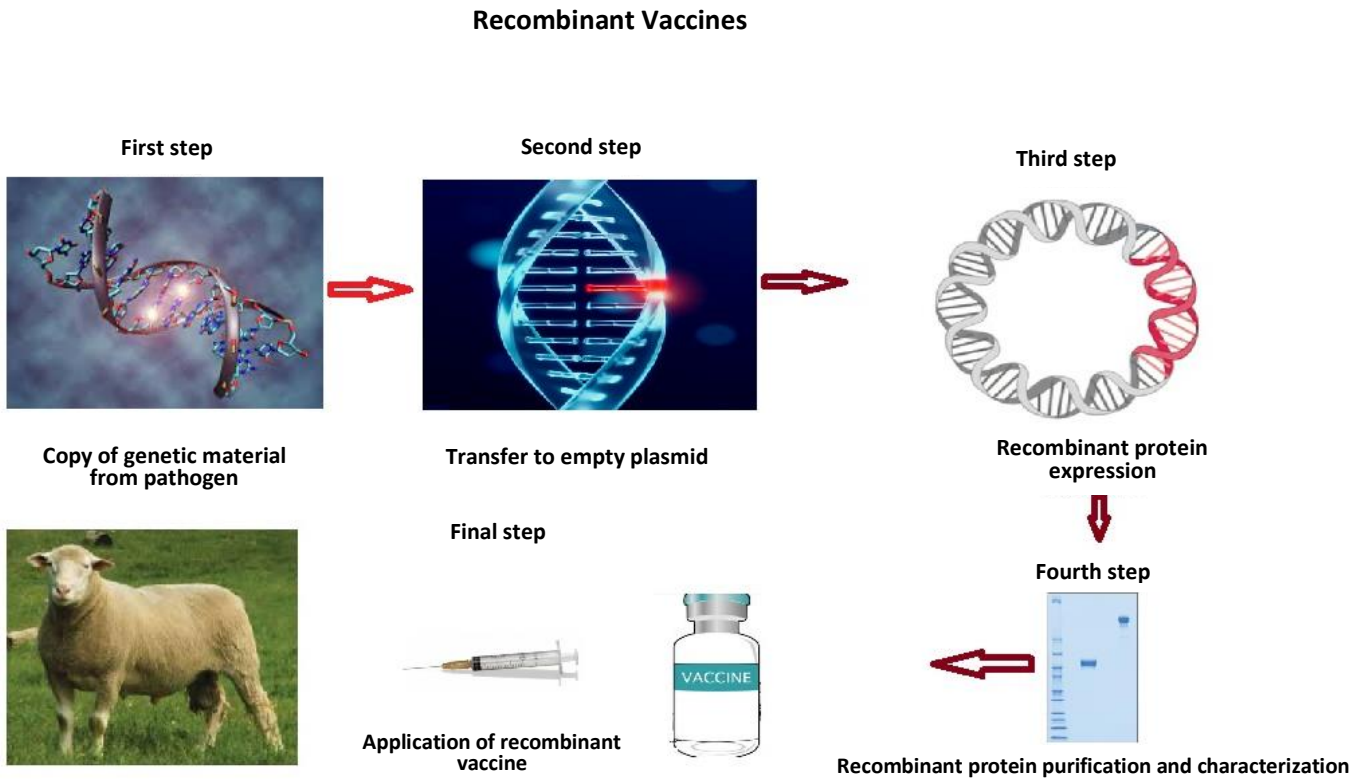

Figure 1. Recombinant vaccine production and application step

Viral Vector Vaccines

Viral vectors are considered potential tools for genetic treatments and vaccinationsThe idea of a viral vector was initially proposed in 1972. Jackson et al. (19) genetically engineered simian vacuolating virus 40 (SV40) to produce recombinant DNA. Vectors' immunogenic power is based on viruses' ability to infect cells (19). Moss et al. (1982) demonstrated the use of vaccinia virus as a transient gene expression vector. In general, viral vectors provide benefits such as (a) extremely effective gene transduction, (b) highly selective gene delivery to target cells, and (c) production of a strong immune response and strengthening of cellular immunity (20). Recombinant viral vectors offer therapeutic potential by facili-

tating intracellular antigen production, prompting robust cytotoxic T-lymphocyte (CTL) responses, thereby eliminating infected cells (21).

RNA Based Vaccines

Vaccinations based on nucleic acid were developed long ago with the intention of establishing a class of vaccines that would be easy to create, safe, and successful. RNA based vaccine study explores two distinct forms of RNA: non-replicating mRNA and self-replicating RNA generated by viruses. Self-replicating RNAs encode both the antigen and the viral replication machinery, allowing for intracellular RNA amplification and profuse protein production. This contrasts with conventional mRNA-based vaccinations, which only encode the antigen with 5' and 3' UTRs. Till the late 2000s, emphasis was concentrated on the development of DNA-based techniques due to challenges such as RNA instability, inefficient immune response when administered in vivo, and promotion of excessive inflammatory reactions. Creating in vitro transcribed (IVT) messenger RNA (mRNA) is a straightforward procedure. Producing high-quality 'therapeutic' mRNA that is very expressible and doesn't cause inflammation has been a major challenge in this field until recently (22). Some critical issues, including the addition of modified nucleosides optimization of coding sequences and high-level purification of IVT mRNA have led to the development of high-performance liquid chromatography (HPLC) to purify RNA contaminants in the early 2010s (23). With this method, the toxicity of synthetic mRNA is reduced by enabling organismal self-detection, and an accurate reading of the mRNA is made possible. mRNA vaccines are a relatively new type of vaccination that shows promise for the future. This strategy is based on newly published research that show the efficacy of mRNA vaccines in treating a variety of malignancies and infectious disorders when traditional immunizations fail to provide protective immunity. Safe, effective materials for in vivo mRNA. transfer and developed protocols for high-quality mRNA production are being studied (22). One of the first COVID-19 vaccines that started clinical trials was the RNA-based vaccine, and studies were initiated with the idea that it would made a significant contribution to the fight against the pandemic of the period (24).

Subunit Vaccines

In the 1950s and 1960s, scientific studies were centered on molecular microbial genetics. In the early 1970s, new information about the role of DNA in cells, the nature of genes, the activity of phages, and the discovery of restriction enzymes led to the alteration of DNA molecules to contain foreign DNA. Recent discoveries in immunology and protein engineering have paved the development path and manufacture of recombinant subunit vaccines. In recent years, numerous approaches have been developed to synthesize recombinant DNA and transfer it to a host cell such as *Escherichia coli* or *Saccharomyces cerevisiae*, or transfer it to a baculovirus-insect cell expression system to produce recombinant proteins (25). During the following years, the focus has been on developing more expression systems with increased power to produce recombinant proteins. This biological revolution resulted in the development of a new concept of vaccines. Working on the notion that safe, inexpensive, and effective vaccine candidates may be created using particular antigens from many infectious agents, a new light was shone on the vaccine concept. The fundamental principle of the subunit vaccination involves isolating the gene encoding the vaccine and transferring it to a second non-pathogenic organism. The heterologous host produces the gene that was introduced to it. The produced gene can be purified and engineered to be administered as an immunogen employing the production host in a living vector or as pure nucleic acids in the form of a vaccine-encoding gene (26).

DNA Vaccines

Vaccines described as third-generation vaccines, genetic immunization or DNA vaccination offer innovative techniques for the prevention and treatment of a variety of bacterial and viral illnesses. DNA vaccines are composed plasmid DNA expression vectors derived from *E. coli* contain genetic instructions for desired antigens, regulated by potent viral promoters recognized by mammalian hosts. Upon injection into an animal, the plasmid DNA prompts the expression of the antigenic gene and antigen-specific immunity develops. To develop a DNA vaccine, the gene encoding the antigen from interest is introduced into the bacterial plasmid under direction of a suitable eukaryotic promoter (27). Because of the nucleotide mismatch among bacteria and eukaryotic cells, single nucleotide polymorphisms typically modify the antigenic genes to increase the effectiveness of expression of genes. The purified and detoxifying plasmid genetic material is then injected into the host animal. Plasmids picked up by suitable cells in the recipient cell cause their own transcription of genes and protein synthesis, resulting in the production of the desired antigen. The host detects the synthesized antigens as foreign and initiates an immunological reaction against it. In the last decade, DNA immunization has emerged as an effective new technique to immunoprophylaxis. It has lately been used successfully to enhance humoral and cellular immune responses in experimental animals and non-human primates (28).

Vaccines Based on Virus-Like Particles

Virus-like particle (VLP)-based vaccinations are a more secure and more efficient alternative to conventional immunizations. VLPs are new fragments of molecules used to guard and control viral illnesses. VLPs are proteins groups that consist of a number of species. Because they lack viral amino acids, they resemble the virus from which they are produced in size and appearance. Recently, many virus-like components have been built using recombination VLP technology approaches. VLPs have also been used as carrier systems to transport foreign antigen epitopes in recently produced candidate vaccines. VLPs were designed for the development of VLP-based plasmodium vaccine candidates (29), diseases caused by group A *Streptococcus* infections (21), Alzheimer's disease (30), allergic asthma, diabetes, tumor and cancer preventive reagents delivery (31). VLP technology opens new pathways for more advanced healthcare uses such as carcinoma immunotherapy, Alzheimer's disease, metabolism and chronic illnesses (32). VLPs are produced through the expression of recombinant proteins. Bacteria, yeast,

mammals, insects, and plants all express viral structural proteins. Eukaryotic cells assemble with empty capsids in vivo, but prokaryotic cells frequently assemble in vitro. More complex VLPs, consisting of multiple viral proteins and a lipid envelope, need an eukaryotes host. An in-depth analysis of the VLP expression systems is necessary for effective production. Many VLP-based vaccination candidates have been generated by advances in genetic engineering, bioengineering, and virus structural determination and these have been assessed in studies in both preclinical and clinical settings. VLP-based immunizations have made major improvements to the fight against cervical carcinoma and hepatitis B infection (33). Even though a variety of adjuvants can be used to improve the immune system reaction in vivo, such as aluminum salts (e.g., the aluminum hydroxide and aluminum phosphate), emulsions made from oil in water (e.g., Span 85 and Polysorbate 80), and AS04 (a blend of monophosphoryl lipid A and aluminum salt), many different adjuvants are used preclinically and/or clinically. Various adjuvants, such as aluminum salts (e.g., aluminum hydroxide and aluminum phosphate), fatty emulsions in water (e.g., Span 85 and Polysorbate 80), and AS04 (a mixture of monophosphoryl lipid A and aluminum salt), can be utilized to boost the immune response in vivo. There are several pre-clinical and/or clinically used additional additives. Modern vaccine design usually considers the selection of a specific adjuvant to stimulate a specific type of immune response (34).

VLP vaccines developed/under development based on recombinant protein;

1.Human papilloma virus (HPV) vaccines

2. Hepatitis B vaccines

3. Hepatitis E vaccine

4. Flu vaccine candidate

5. Norwalk virus candidate vaccine

6. Ebola and Marburg virus vaccine candidate

7. Hepatitis C candidate vaccine

8. Human immunodeficiency virus (HIV) vaccine candidate

9. Malaria candidate vaccine

Marker Vaccines

Throughout history, vaccines have been developed to prevent disease or reduce the severity of clinical manifestations of infections. However, vaccination can sometimes interfere with serologic diagnosis and determination the prevalence and incidence of infection when the antibody response after vaccination is indistinguishable from the immune response after infection. Marker vaccines have provided a solution to this issue. Using a diagnostic kit, a marker vaccine is a sort of vaccination that makes serological discrimination between vaccinated and infected animals easy but reliable. A marker vaccination is a vaccine (inactivated or live) based on delete mutations or identified proteins from bacteria to distinguish vaccination and infected individuals were separated based on adequate antibody responses. This vaccine is used in conjunction with a test that identifies antibodies against a protein that is absent in the vaccine stem (35).

A proposed marker vaccination must meet a few minimum requirements (36).

1. It should not cause immediate or prolonged the risks in vaccinated animals.

2. Not pose a risk to immunized animals or different species following genetic recombination.

3. Be simple to produce following a consistent methodology.

4. Develop permanent immunity immediately.

5. A completely immunity to all known versions of the virus should be developed.

6. The agent should not be transmitted vertically or horizontally.

7. A simple yet extremely accurate and specific differential diagnostic test should be available.

The first step in candidate marker vaccine design should be create an efficient examination for diagnosis. In this respect, the phrase "marker vaccine" is not a very clear idea, because the primary distinction between marker vaccines and standard immunizations is the ability to distinguish between vaccinated and infected animals' antibody responses. Therefore, a marker vaccine can be referred to as a difference of infectious field strain from vaccinated animals (DIVA) vaccine (37).

Generally, there are three marker vaccination techniques: (38)

1. Marker vaccine is a strategy as a "negative marker" by excluding a minimum of one immunological epitope or protein when the field strain and vaccine strain are compared.

2. "Exogenous positive marker vaccine" by involving an immunodominant antigen or protein not normally present in a potent vaccine.

3. "Intrinsic positive marker" is a marker vaccine created by involving an epitope or immunogen present in the causative agent but stimulates a different antibody response than the vaccine strain of the field strain.

So far, practically all potential marker vaccines have been developed using a "negative marker" technique. Serologic diagnostic methods produce results by testing antibody against the target protein, which is not present in the marker vaccination. Animals that tested positive had been infected. Aside from technical issues, their specificity and sensitivity are mostly influenced by the immune system's response to the potential vaccine and natural infection. The subsequent production of antibody against negative markers, or the low number of such animals following a spontaneous infection, might significantly reduce the DIVA potency of the diagnostic test. In theory, a marker vaccination containing only negative indicators is sufficient to elicit a different antibody response than spontaneous infection. If the marker vaccination includes positive and negative markers, infected or suspected animals can be discriminated from vaccinated ones more efficiently. Vaccination with a marker vaccine is an effective strategy for limiting the magnitude of infectious outbreaks (39). Serological testing can be used to diagnose immunity after receiving marker vaccinations. Incidence and frequency can be calculated among vaccinated persons. The vaccine's efficacy can be tested, allowing the vaccine to be used in conjunction with an eradication program (40).

Table 1. Advantages and disadvantages of vaccine types

Vaccine Type	Advantage	Disadvantage
Recombinant Protein Vaccines	Protects against multiple antigens with excess protein production.	Adequate immunogenic response may not always be achieved with a single protein and therefore effective adjuvants should be used.
Viral Vector Vaccines	Recombinant viral vectors, like natural infections, actively boost immunity and have innate adjuvant properties.	To produce viral vectors, appropriate cell lines must be propagated. This raises the cost of production.
Subunit Vaccines	Its structure is more stable, safer than attenuated vaccines and more suitable for large-scale production.	Their benefits are restricted by promoting humoral immunity because they are not replicative, cannot adequately induce the cellular immune response, and are supplied externally to the antigen-presenting cell.
DNA Vaccines	Increases an effective and long-lasting cellular immunological response.	It may not always provide sufficient immunity.
Vaccines Based on Virus-Like Particles	It generates a relatively better immune response due to the presence of multiple epitopes that better simulate the surface of viruses and microorganisms	Difficulties in the production process
Marker Vaccines	It works based on deletion mutants or isolated microbial proteins that allow discrimination between vaccinated and infected individuals based on their respective antibody responses.	The late emergence of antibodies against negative markers, as well as the low percentage of such animals following natural infection, can significantly decrease the diagnostic test.

CONCLUSION

Human beings, who have been struggling with epidemics that have been a major problem for humanity for centuries, have sought ways to fight even when they could not identify the disease agent. It has been seen that the most effective way in this war is related to immunization against that agent. Synthetic immunity and protection against an agent is provided by vaccines. Vaccines have always been developed to provide more effective and longer-lasting immunity since they were first developed. Traditional vaccines, which first emerged with the approach of isolating, inactivating and injecting the agent, have been tried to be made even more effective and harmless over time and with advancing technology. With the discovery of recombinant DNA technology, second and third-generation vaccines emerged. With the advancement of genomic studies, vaccines using nucleic acid, not the causative agent, have been produced. With the reflection of these advances in vaccine studies, vaccine science has reached a point where it is more effective, safer and less costly. Today, access to vaccines has become easier. The number of people who have died and been affected by the COVID-19 pandemic worldwide is enormous. Therefore, the most important issue that scientists focused on was vaccine studies. Thanks to the products of these studies, the pandemic ended. It is seen that preventing a disease is more effective than treating it. In this context, vaccines are our indispensable weapons. Every day, all the work is going on to make these weapons even more effective. With advancing technology, studies continue at full speed to make vaccines, our most valuable defense tool against infections, even more effective.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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