An insight on Recombinant DNA Vaccine: A New Era for Development

Introduction

Human lives are mainly affected by the quality of food, health and environment. For a stable lifestyle maintaining health and having good food are most crucial things. The recombinant DNA technology plays a vital role in improving healthcare by the development of new vaccines and pharmaceuticals. To improve healthcare, new strategies are involved which help them to enhance the organism capabilities, by altering their genetic material, and then expressing them to obtain the desired characters or products.

Recombinant DNA technology comprises the genetic material to enhance the desired characteristics of any organism which will work by producing a product. This technology involves insertion a desired DNA fragment which possess desirable characteristic and they can bind with a specific vector. Manipulation of the genes can be carried out by either expressing different desired genes or by repressing the endogenous genes. Restriction endonuclease enzymes are used for cleaving of DNA fragments for specific target sequence, followed by DNA ligase to fix the desired gene into a vector system. A vector is introduced into host system which allows them to clone and expressing their desired characteristics (Khan et al., 2016).

Recombinant DNA technology is a rapidly growing filed for scientist and researchers around the globe for developing novel devices, and engineered products for the application in agriculture, health and environmental sectors. Recombinant DNA technology have a broad spectrum of applications in various sector like therapeutic products such as vaccines, antibodies, growth hormones, vectors, recombinant proteins; genetically modifies products including fruits, vegetables, crops and microbes; for diagnosis and various energy utilization function. R-Technology has wide application if treating diseases and improving health. By various immunodominant antigens, immune protection against various disease can be achieved. In compared to traditional vaccines, which have a lower efficacy and specificity, recombinant vaccines are in rapid demand for new approach to provide immunity and specificity against any disease. But the challenge is to make a stable vaccine, which have rapid biological effect, cost-effectiveness, biological safe, and transversal in nature for large scale production (Khan et al., 2016, de Pinho Favaro et al., 2022).

History of Vaccines

Vaccines are biological product which have been safely administered to provide protection against any disease, or pathogen. They generally include one or more antigens or proteins which produces an immune response (Giese, 2016; Pollard & Bijker, 2021).

Vaccination is a biological process in which the body is exposed to a biological stimulus as live attenuated or a killed organism, a subunit of organism, or a DNA/RNA encoding the antigenic product against that pathogen to provide protection generating an immune response. Vaccination represents a single most cost-effective way to fight against any infectious disease. (Kozak & Hu, 2023)

The word vaccine came from the Latin word Vacca meaning cow, and vaccinia is cowpox, so he named his experiment as vaccination (Riedel, 2005). Vaccine term was understood recently, but the concept of immunization was known long back with Edward Jenner's discovery against cowpox immunization, who found out that milkmaids who were exposed to cowpox were immunized against the much smallpox disease. Till date the live vaccine virus is only given as vaccine for smallpox (Kozak & Hu, 2023). Three main vaccines are approved for smallpox which includes Dryvax the first-generation vaccine, which is replaced by ACAM2000 a second-generation vaccine with same efficacy and safer to use. The MVN-BN is third generation vaccine which is currently approved by FDA for its most stable and safer profile in compared to other two vaccines (Saleh et al., 2021)

The development of vaccines then started with pasture who took the cerebrospinal fluid homogenates of rabies-infected rabbits and separated the live attenuated to inactive virus for rabies vaccine. Simultaneously around the same time Sawtschenko and Sabolotny also worked on heat killed vibrio cholerae bacteria, and later formed the first whole-killed vaccine for oral vaccination. Another development in the vaccines were of toxoid vaccine against diphtheria and tetanus which were formed when diphtheria was inoculated in horse, and their sera was isolated. This exposed toxoid was mixed with active diphtheria toxin and was administered as a dose to generate immune response and allowing the system to develop a vaccine response. In present times, toxoid vaccines are neutralized by formalin inactivation (Kozak & Hu, 2023).

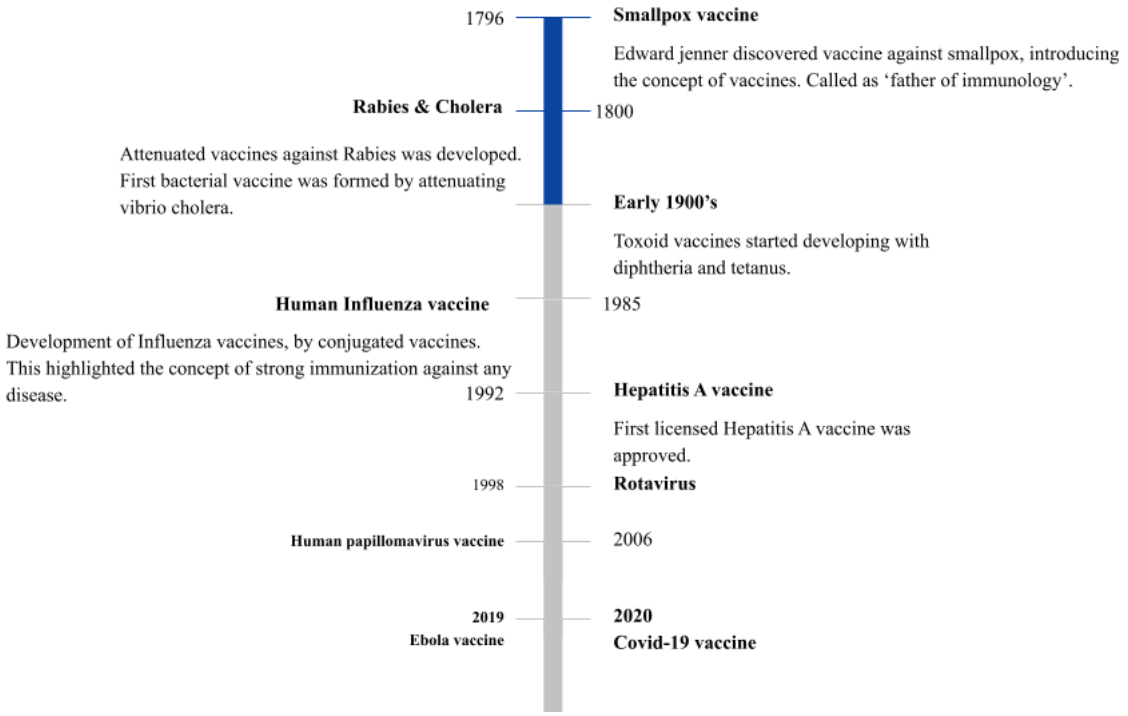

Figure 1. Timeline for vaccine development.

With the development and awareness in cell culture, the experiments with virus and vaccine development started, and lead to the discovery of influenza vaccine. Influenza A and B virus had segmented genome along with the identification of neuraminidase and hemagglutinin which when co-cultivated and colonies were formed and identified as harmless virus, creating

live and inactivated influenza vaccines. (Kozak & Hu, 2023) Rotavirus is main causative agent for acute gastroenteritis, and since the discovery of this virus, vaccine development started. The first FDA approved vaccine is Rotashield in 1998, but it was withdrawn. In 2006, another vaccine RotaTeq was approved and now Rotarix- another FDA approved vaccine is most used vaccine against Rotavirus (Saleh et al., 2021).

With the shocking discovery of influenza vaccine, there was a concept well understood that passaging the virus strain with many hosts can weaken the virus, which came up with the new concept of live attenuated vaccines. Polio, measles and rubella were the first amongst the development of live attenuated viral vaccines. Genetically developed vaccines were formed much later with Hepatitis B vaccine being the first subunit vaccine, as the HBV could not replicated in cell culture, and it had to be inoculated with hepatitis b surface antigen into a yeast plasmid and then developing the first recombinant vaccine. (Kozak & Hu, 2023). Human papillomavirus is most common cause for cervical cancer in women. FDA in 2006 approved the first HPV vaccine, and since then the cause for cervical cancer and morbidity has reduced (Saleh et al., 2021)

Immunization:

With the vaccine development the term immunization is known to provide protection against any disease. There are two different types of immunization which is passive and active immunization, which occurs through natural or artificial factors. (Clem, 2011)

Passive immunization meaning the transfer of antibodies to any unimmunized individual, which would develop a temporary immune response to a particular toxin or disease. Once the antibodies are used against the infection, they would no longer be able to generate the immune response. Best example of passive immunization is the transfer of antibodies from mother to foetus through placenta and infant through colostrum and milk and the administration of human immune gamma globulin and antivenin. (Clem, 2011)

Active immunization occurs when the unimmunized individual is exposed to a pathogenic agent, the immune system will begin the process of developing the immunity against the pathogen. In contrast to passive immunization, active immunization will produce a long-term immunity. (Clem, 2011)

Types of vaccine:

Inactive vaccine:

Whole pathogen vaccines are generally the traditional vaccines, as they came up with the concept of killing or weakening of pathogens which cannot cause disease. They include inactivated or killed pathogens as vaccines, as they can create a very strong immune response. Inactivated or killed vaccines are prepared by inactivating the pathogen by heating, radiation or use of any chemical agents like formalin and β-Propiolactone. By inactivating any virus, it will not allow them to replicate in human cells but will keep the antigens alive in the viral structure, allowing various immune response. They have generally low efficacy rate, and they

require adjuvants for their efficient immune response. Vaccines for Influenza virus, hepatitis A virus, rabies virus has been successfully prepared through this vaccine strategy (D'Amico et al., 2021; Sanders et al., 2015).

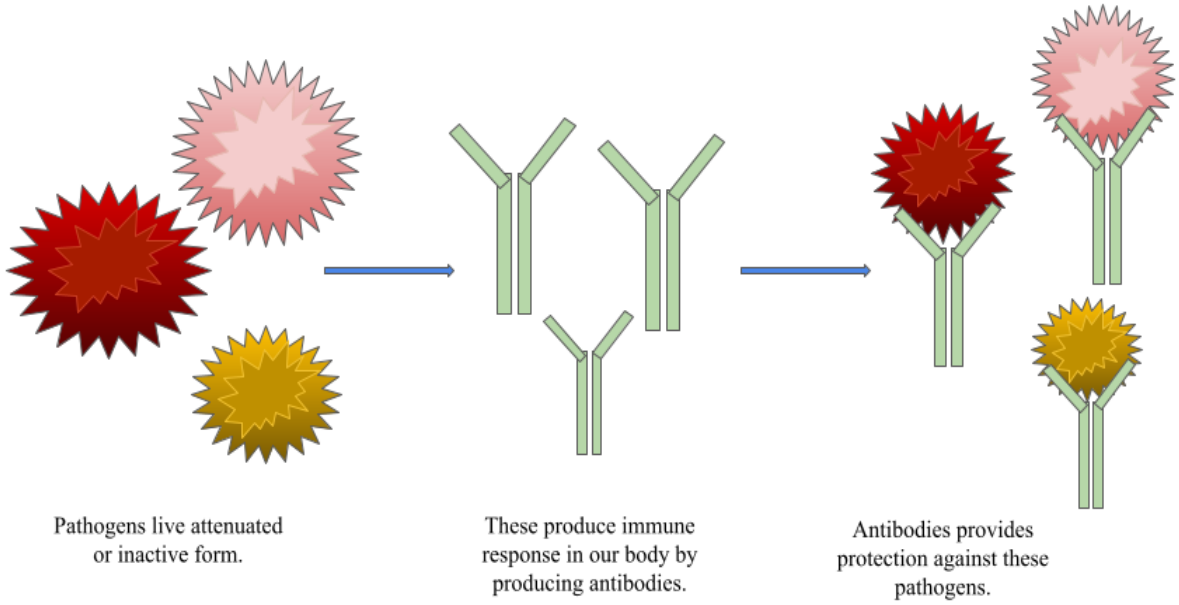

Figure 2: Illustrative for live attenuated and inactive vaccine formation.

Live attenuated vaccines:

As many of the pathogens which are inactivated cannot generate immune response, another concept was introduced where living pathogen can be weakened in such a way that it cannot cause disease. Live attenuated are the vaccines against many human diseases such as polio, measles, smallpox, mumps, rotavirus, and yellow fever have been completely eradicated and have been most successful, cost-effective inventions of medical history. Attenuation is a process by which a pathogen is passed through the series of host and its pathogenicity is reduced. Attenuation can be a robust method for the vaccine production and generating a strong immune response. The main advantage over attenuated vaccines is that they maintain the nature of antigens and mimic the natural infection by establishing an immune response (Kozak & Hu, 2023). They are usually closely related virus which are non-pathogenic for human, or have less virulent strain, or are obtained from repeated subculturing in cells. Live attenuated vaccines are easy to produce for virus, then in compared to complex bacteria and pathogens. As these vaccines contains a live attenuated viral strain, they are usually temperature sensitive, and stored at cold conditions (D'Amico et al., 2021; Minor, 2015).

Subunit vaccines:

Subunit vaccines are also acellular vaccines which contain purified pieces of pathogens which have ability to stimulate an immune response (Kozak & Hu, 2023). For subunit vaccines, as the name suggests instead of whole pathogen, only components or antigens that can stimulate best immune response are used. They are easily producible and safe to use, and as they are subunits, they often require adjuvants to elicit a strong protective long-term immune response.

They can include toxins and carbohydrates as the subunits and produce toxoid or conjugated vaccines. Toxoid vaccines are another type of vaccine prepared from toxins secreted from bacteria like tetanus and diphtheria, which are commonly administered worldwide. They are not immunogenic, and thus an adjuvant is required for the immunogenic response, but they are highly stable vaccines. Conjugated toxoid vaccines are also most common as they are conjugated with polyglucans and proteins to generate a strong immunogenic response (D'Amico et al., 2021).

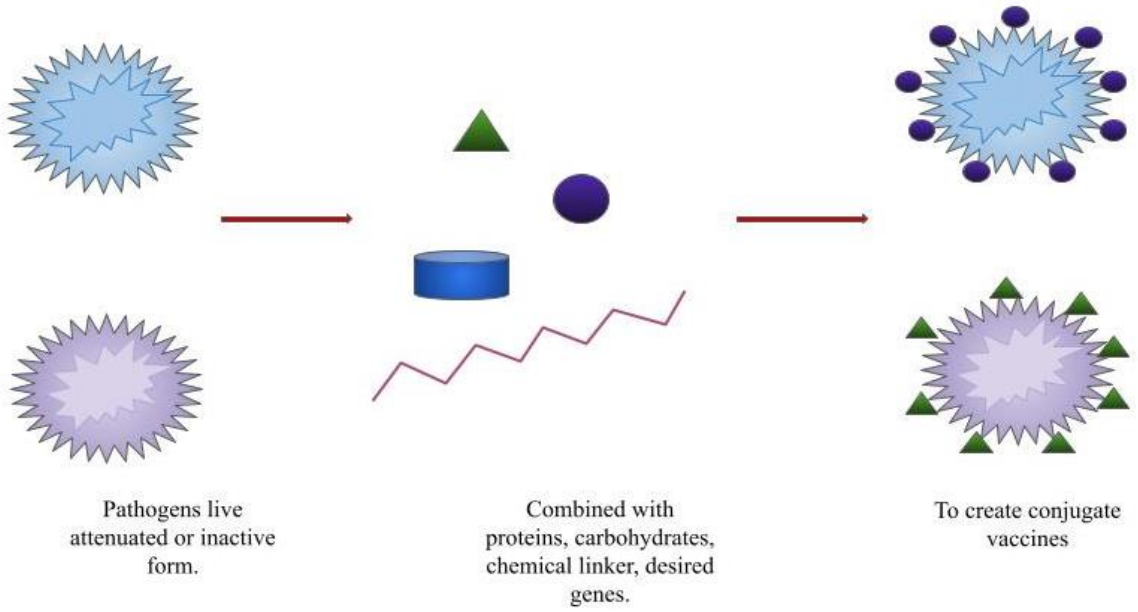

Figure 3 Illustrative for subunit & conjugated vaccine.

Recombinant vaccines

Many vaccines approved are having whole virus or pathogen cells in inactivated form or live form, but now-a-days due to recombinant technology there are a shift of vaccine production towards exploring this technology and producing a protective vaccine. As the advances in laboratory, genetic engineering become very useful for vaccine production which led to a new era of modern vaccines, recombinant vaccines. The need for recombinant vaccine is increasing as it can increase protective effectiveness and can be economically easy to be manufactured. There are many trends for recombinant vaccines including nanovaccines, nucleic acid vaccines, and protein subunit vaccines by using various expression systems (de Pinho Favaro et al., 2022).

Protein and subunit vaccines

The recombinant vaccines can be produced by using an overexpressed protein from a genetically modified strain, to link a subunit vaccine by using recombinant technology. The hype for recombinant technology meant that foreign genes could easily be inserted into expression vectors, and introduced into cells, for the expression of their desired characteristics.

These vectors can be easily introduced into a cell that can act as a production factory, for relatively cheap source of desired protein encoded from the cells, to produce recombinant vaccines by using bacterial, mammalian, yeast and insect cell as an expression system (Francis, 2018, de Pinho Favaro et al., 2022).

Bacteria are used as a choice for heterologous protein expression system through recombinant DNA technology. E. coli bacteria is used as the first ever bacterial recombinant expression system for producing large quantities of defined proteins used for subunit vaccines. It is mostly recommended due to its fast growth rate, different engineered strain, and easy for production.

As E. coli is prokaryotic cell having different mechanism for processing and expression of protein folding differently, there are chances that the desired protein might be folded incorrectly resulting in errors during the post translational modifications, causing toxicity, insolubility, or rapid degradation of protein into bacterium system. This can be a disadvantage for vaccine production in bacterium system, but the first recombinant veterinary vaccine was produced by using E. coli expression system (Francis, 2018).

Saccharomyces cerevisiae, commonly known as baker's yeast, an industrial microorganism, has become natural alternative for the antigenic proteins expression system, as it has similar type of post translational modification as of higher eukaryotes avoiding the misfolding of proteins. These expressed proteins can be glycosylated through glycosylation carried on the mammalian cells. Another potential strain of yeast Pichia pastoris, has a 10-fold high levels in compared to S cerevisiae, has been used as an expression system to express Human hepatitis B vaccines based on virus antigen levels as high as 400mg/L (Francis, 2018).

Another novel system has been developed by using insect ovarian cells from Spodoptera frugiperda infected with a baculovirus vector Autograph californica nuclear polyhedrosis virus. They possess a strong promoter that controls the production of 29 kDa polyhedron protein almost 50% of total cell protein, and by replacing the polyhedron head with a foreign gene, high levels of desired protein can be produced (Francis, 2018).

As many pathogens can infect and replicate only in mammalian cells, another expression system for authentic protein for subunit will be developed in mammalian system. The proteins present and expressed have been found to have lower expression levels in this system as when compared to alternative system. Still many vaccines protein for Bovine viral diarrhoea (BVD), VLPs for Japanese encephalitis virus have been produced (Francis, 2018).

Nucleic acid vaccines

Nucleic acid vaccines generally used genetic material such as DNA or RNA from pathogen to introduce an immunogenic response in host cells (Kozak & Hu, 2023). Nucleic acid vaccines are usually plasmids- a circular piece of DNA, which many contain a foreign gene to express a protein in target animal. These plasmids can be maintained in bacteria, and they are designed in such a way that it can accept any foreign gene for its expression in animals. These recombinant plasmids contain a foreign gene will have to be purified from bacteria and this naked DNA will be inserted into host via intramuscular or intradermal route, which will

generate an immune response via the protein expressed by foreign genes. The CpG motifs embedded in DNA is a unique feature for DNA vaccines, which are unmethylated motifs which act as an adjuvant and helps in stimulating innate immune response and enhances the vaccine effectiveness (Jackwood, n.d.). Nucleic acid vaccines have been developed because of good stability, fast production, safety profile and enhanced immune response.
Main advantage of nucleic acid vaccines are they have no live pathogen and thus aren't capable of causing any native disease. They are also rapidly modified and can be a promising therapeutic agent. Mode of action for DNA vaccines is tricky as they when introduced into cells will require mRNA processing and then can be used, while mRNA vaccines will already have post translational processing and can be immediately used. Thus, mRNA vaccines provide a major advantage over DNA vaccines. Recently, two mRNA vaccines and one DNA vaccines has been approved by FDA against SARS-CoV 2 virus. (D'Amico et al., 2021; Kozak & Hu, 2023).

Nanovaccines

The traditional vaccines are plain, subunit vaccines are considered safe but less immunogenic as they lack PAMPs, thus may require adjuvant for it to fully function. Nonvaccine is a novel approach that combines an adjuvant with nanoparticle which is conjugated with an antigen, or it also acts as a carrier for DNA, RNA and recombinant proteins. Various antigens can be conjugated with nanoparticles, in order to generate immune response against all of them. Nanovaccines can activate both cellular mediated and antibody mediated immunity, in turn proving to be a cost-effective option. Nanovaccines provide extra protection against the degradation of the vaccine components and also protect from cleaving enzymes. Nanovaccines can also be designed to target a specific cell type or tissue in order to avoid the side effects on other cell types. Multiple antigens can be entrapped with a single nanoparticle and a broad range of immune response could be generated against the antigens (Azharuddin et al., 2022; Yin et al., 2022)

A variety of nanomaterials has been reported which can be used in nanovaccine. The most efficient one is noble gold particles due to its non-toxic and biologically inert characteristics. Gold nanoparticle nanovaccines have widely been reported against influenza, foot and mouth diseases, cancer, and malaria. In addition to gold nanoparticles, polylactide-co-glocolic acid (PLGA), polystyrene and chitosan are also seemed to present or carry an antigen in case of nanovaccines against Hepatitis B virus, bovine serum albumin and nicotine. The mode of action of nanovaccines is also simple. In order to elicit an immune response, the antigens which are conjugated with nanoparticles will first be acquired by antigen presenting cells (APCs) and their antigenic peptides will be presented by MHC-I or MHC-II. APCs will also release various pro and anti-inflammatory cytokines. In addition to this, clonal expansion of B cells and T cells will also initiate, and B cells will start releasing the antibodies against the specific antigen. Thus, from a single nanovaccine both humoral and cellular-mediated immune response will be generated (Chen et al., 2010; Gutierro et al., 2002; Kanchan & Panda, 2007; Rodriguez-Del Rio et al., 2015; Tao et al., 2014; Zhao et al., 2017)

A number of nanovaccines have been under clinical trials and some of them have successfully completed. ResVax^{TM} by Novavax has successfully completed the trials for ResVax^{TM} and EBOV GP against respiratory syncytial virus (RSV) F protein and Ebola virus

respectively. Sensei biotherapeutics launched PAN-301-1 against prostate cancer and trials are over. DPX-0907 by ImmunoVaccine Technologies has also finished the trials against ovarian, breast and prostate cancer. Another one belonging to the list is Tecemotide by Merck against multiple myeloma. ONT-10 by Cascadian Therapeutics and Lipovaxin-MM by Lipotek Pty are also registered for solid tumors and metastatic melanoma. Development of nanovaccines during the SARS-CoV2 pandemic took a hit. A wide range of vaccines were launched by pharmaceutical companies for covid-19 protection. A few of the popular ones are mRNA-1273 LNP by Moderna and NIAID, mRNA BNT162b2 by BioNTech and Pfizer, NVX-CoV2373 by Novavax, LNP-nCoV saRNA and ARCT-021 by Imperial College, London Arcuitas Therapeutics. These vaccines targeted m-RNAs, sa-RNAs and spike proteins. Apart from these, nanovaccines against HPV have also been reported which includes Gardasil®9 by Merck and Cervarix® by GSK (Azharuddin et al., 2022)

Conclusion and Future Scopes:

Recent advancements in the field of recombinant DNA technology have made the scientific community to expand our knowledge about vaccine development. The biggest advantage of using r-DNA technology in vaccine development is that one can design a specific vaccine which can mimic the exact mechanism by which a viral pathogen attacks the immune system and thus can have a better immunization against the pathogen. Needless to say, it also comes with the side benefits like cheaper costs, easier to handle and transport and enhanced stability along with less toxicity. The development of third generation vaccine still requires having extensive research. In order to continuously enhancing the immunization, a variety of adjuvants and their mechanism of action needs to be studied. Designing a vaccine through r-DNA technology also requires a broad understanding of the mechanism of a disease. This brings certain restrictions to develop a vaccine in case of pandemics where the cause, mechanism of infection and target pathogen is unknown. Thus, large-scale research is required to design and manufacture the vaccine in an efficient and faster manner. The field of vaccine development for immunocompromised individuals is still in an infant phase and in this case, designing the vaccines through r-DNA technology should be explored to elicit the immune response against the pathogen without provoking and targeting body's own immune system. More research should be done to focus on delivery routes other than subcutaneous and intramuscular. Additionally, role of vaccines as a therapy should also be investigated. Cancer vaccine is an astonishing achievement in the field and designing vaccines against other diseases is also needed. These opportunities and challenges can bring a major change in the field of vaccine development through r-DNA technology and will ultimately improve human lives.

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