Journal of American Science

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Multidisciplinary Academic Journal Publisher

Vaccination has been an important tool to combat infectious diseases

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ABSTRACT: Development of vaccination as a tool in fighting disease has resulted in the potential to combat almost all infectious agents affecting people and animals. The ultimate objective of vaccination is to induce an immune response that subsequently recognizes the infectious agent and fights off the disease. Recombinant DNA technology is any technological application that uses biological systems, living organisms' derivatives to make or modify products or processes for specific use. Using recombinant DNA technology scientists are working vaccines. Recombinant DNA technology has indeed made tremendous breakthrough in the discovery of various vaccines. The new generation vaccines prepared from the viral or microbial proteins, their fragments or nucleic acid sequences have been attractive because of their stability, non-infectious nature, homogeneity as well as their cost effectiveness. This paper provides a brief historical overview of vaccine development and describes three basic categories of newer, recombinant vaccines: live genetically modified organisms, recombinant inactivated (killed) vaccines and genetic vaccines.
[Aderaw Desta and Marie Tesahger Tsehay. **Vaccination has been an important tool to combat infectious diseases.** *J Am Sci* 2025;21(4):21-31]. ISSN 1545-1003 (print); ISSN 2375-7264 (online). http://www.jofamericanscience.org. 04 doi:10.7537/marsjas210425.04

Keywords: vaccine; recombinant DNA technology; DNA vaccine; DNA

1. INTRODUCTION

Vaccination has been an important tool to combat infectious diseases over the past 200 years. The first human vaccine was developed in 1798 when Edward Jenner successfully prevented smallpox infection in milkmaids (Makela, 2000). Vaccination is aimed at inducing active immunity in an individual, so that subsequent contact with the microorganism following natural infection induces strong protective immune response. The protective immunity may involve secretion of neutralizing antibodies or production of memory cells. The use of vaccines is now being extended to immunize against tumors or to block fertilization (contraceptive vaccines). A vaccine is a suspension of whole (live or inactivated) or fractionated bacteria or viruses that have been rendered nonpathogenic, and is given to induce an immune response and prevent disease. Even though no vaccine is entirely safe or completely effective, their use is strongly supported by their benefit-to-risk ratio (Sridhar, 2006).

Vaccine is the use of biological preparation for immunizations. Vaccines represent an invaluable contribution of biotechnology and provide protection against various diseases (Rakesh, 2007).It has a prime role in controlling and eradicating lot of dreadful diseases both in animals and humans throughout the world. Except the term vaccine nothing is constant from the era of Edward Jenner, who first immunized James Phipps against small pox in 1796 (Stewart and Devlin, 2006).An ideal vaccine formulation should consist of following features:-It should be safe with minimum side effects, eco-friendly, produce long lasting effective humoral and cell mediated immunities, simple to administer, cheap to be affordable to all the classes of people and not be toxic. (Sridhar, 2006; Rakesh, 2007). Construction of vaccines has undergone lot of mutations starting from live attenuated to the gene based vaccines. Over the years knowledge about the different corners of the vaccine has been to the limelight based vaccines. Now-a-days every immunological aspect, mechanism of

vaccine action is clearly unearthed (Kumaragurubaran and Kaliaperumal, 2013).

The use of vaccines has had great impact on the ability to control and prevent infectious diseases. The first vaccines consisted of whole pathogens, killed or attenuated, but today the recombinant subunit approach, that is to use only a defined subunit of the pathogen, is dominating the vaccine research in the search for new effective vaccines. The ability to use small, defined parts of a pathogen and produce that subunit in a non-pathogenic host will increase the safety of future vaccines. Subunit vaccine candidates typically consist of surface proteins or polysaccharides. The use of recombinant DNA technology has simplified the production of subunit vaccines. Protein-based subunit vaccines can consist of synthetic peptides, recombinant proteins or gene fragments encoding the protein, immunogens. Live delivery vehicles can furthermore be evaluated for delivery of both protein subunits and nucleic acid vaccines (Christin, 2000).Recombinant DNA is created by combining DNA sequences that would not normally occur together in nature (Garret and Grisham, 2008). In appropriate conditions a recombinant DNA molecule can enter in cell and replicate there (Jonathan and Wiley, 2003). It differs from genetic recombination, in a way that it does not occur through processes within the cell, but is engineered (Garret and Grisham, 2008). Using recombinant deoxyribonucleic acid (rDNA) technologies, scientists have been able to develop three types of recombinant vaccines: live genetically modified organisms, recombinant inactivated ("killed") vaccines and genetic vaccines. These vaccines no longer cause disease, but still induce a strong immune response. Paralleling the development of new, more efficacious, stable, and safe recombinant vaccines has been the study of vaccine delivery methods and immune stimulating adjuvant compounds that enhance the immune response (CAST, 2008).
Therefore, the objective of this paper is:
To review on vaccine development using recombinant DNA technology

2. LITERATURE REVIEW

2.1. History of vaccine development and recombinant DNA technology

2.1.1. History of Vaccine Development

The year 1996 marked the 200th anniversary of the first vaccine developed against smallpox by Edward Jenner. Two months later, he scratched James again, this time with small pox virus. The rest is history: James Phipps did not come down with small pox. Since then, the pioneering work of Louis Pasteur, Albert Sabin and Jonas Salk has led to the development of vaccines against diseases such as rabies and polio. This empirical approach to vaccine development led to the development of first generation vaccines which essentially consisted of attenuated, live or killed pathogens as vaccines (Rangarajan, 2002). Conventional vaccines consist of whole pathogenic organisms which are either killed or live but its virulence is greatly reduced (attenuation). It suffers various limitations although it is relatively easy to produce at low cost. It carries a risk of disease due to the occasional presence of active virus particles or reversion of virulence after one round of replication in the vaccinated individuals (Rakesh, 2007).

2.1.2 History of recombinant DNA technology

Conventional techniques of dealing with livestock diseases comprised of vaccination, slaughter of infected stock, chemotherapy and other management practices. But these techniques are often
unaffordable for the farmer including conventional vaccines because pathogens often become resistant to them. The solution of this bottle neck is recombinant vaccines produced by recombinant DNA technology (McCullough, 1993). These vaccines are for life time with some booster after sometime (Reid, 1989). Development of recombinant biotechnology date back to 1953, when double helical structure of DNA was elucidated by Watson and crick and the genetic code was cracked by Nirenberg. Cohen and Boyer in 1973 invented the technique to cut and paste DNA sequences that is the concept of restriction enzymes came into the picture. Since then recombinant DNA technology has rapidly progressed and expanded. Regions of DNA called genes were found to contain information

that would lead to synthesis of specific proteins, which are strings of amino acids. Each of the protein is unique in context of its function and the reaction it catalysis. If now one is able to express a natural gene from any organism in a very simple bacterium such as Escherichia coli, a bacterium living in intestines that has become the model organism for biotechnology and brought a turning point in the field. Now, one can induce this bacterium to make a lot of protein that is coded by the gene regardless of the nature and source of donor organism (Rakesh, 2007).

The first miracle of recombinant DNA technology was the production of Human insulin. The specific gene sequence, or oligonucleotide, that code for insulin production in humans was introduced to a sample colony of E. coli. In 24-hour period, there may be billions of E. coli that are coded with the DNA sequences needed to induce insulin production (Johnson,1983).

2.2. Genetic engineering, Recombinant DNA technology and Cloning

Genetic engineering is the process of taking genes and segments of DNA from one species and putting them into another species, thus breaking the species barrier and artificially modifying the DNA of various species. These procedures are of use to identify, replicate, modify and transfer the genetic material of cells, tissues or complete organisms (Izquierdo, 2001). The techniques are
generally related to the direct manipulation of DNA oriented to the expression of particular genes
(Hugon, 2006). Techniques in genetic engineering include; the isolation, cutting and transfer of specific DNA pieces, corresponding to specific genes (Klug and Cummings, 2002). Recombinant DNA (rDNA) is defined as a DNA sequence artificially obtained by combining genetic material from different organisms, as is the case for a plasmid containing a gene of interest (Rossana and Cristina, 2010). Recombinant DNA Technology is strongly associated with cloning. Cloning is the process of producing populations of genetically-identical individuals asexually. Cloning in biotechnology refers to processes used to create copies of DNA fragments (molecular cloning), cells (cell cloning), or organisms. Recombinant DNA technology produces the initial cell from which the host organism is then expected to recapitulate when it undergoes further cell division (Jeremy *et al*., 2002).

Gene cloning is the main tool of the recombinant DNA technology. Gene cloning and vector construction are widely applied techniques in r-DNA technology and protein research and are the most frequently used technologies in a molecular biology laboratory. In fact, to study a particular gene, the first step is usually to clone and express it (An *et al*., 2007). There are many approaches available for DNA cloning. The classical technique generally involves cleaving a destination plasmid and a target insert sequence with restriction enzymes, and then stitching them together with the help of DNA ligase. This approach is enormously convenient and straight forward when the appropriate restriction sites are well positioned in the sequences being manipulated, but becomes problematic when these restriction sites are not present. Additionally, most of the eukaryotic genes are interrupted by intervening sequences (introns), which make the gene of interest very large. Manipulation of the large genomic DNA is tedious and problematic due to size capacity of cloning vectors and multiple restriction endonucleases which make it difficult to find appropriate enzymes for subcloning (Sambrook and Russell, 2001).

2.2.1 Basic Steps of Gene Cloning

A fragment of DNA, containing the gene to be cloned, is inserted into a circular DNA molecule (vector) and gives "Recombinant DNA molecule". The vector acts as a vehicle that transports the gene into a host cell (usually, bacterium) resulting, possibly other types of living cell. Within the host cell the vector multiplies, producing numerous identical copies not only of itself but also of the gene that it carries. When the host cell divides, copies of the recombinant DNA molecule are passed to the progeny and further vector replication takes place. After a large number of cell divisions, a colony, or clone, of identical host cells is produced. Each cell in the clone contains one or more copies of the recombinant DNA molecule. Finally the gene is cloned (Seungwook, 2004).

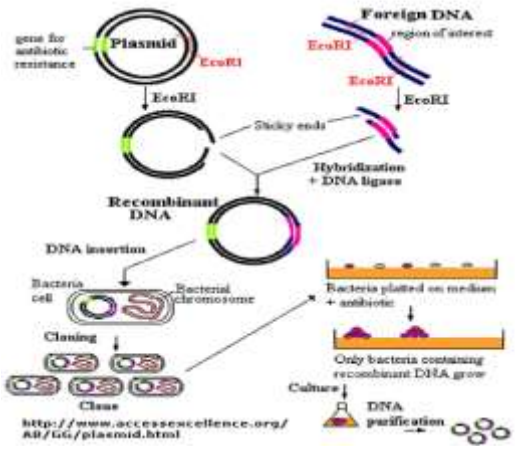

Figure 1: Cloning in to plasmid

2.3 Types of recombinant DNA vaccines

Recombinant vaccines fall into three basic categories: genetic vaccines, recombinant inactivated vaccines and live genetically modified organisms, (Ellis, 1999).

2.3.1 DNA Vaccines

Genetic or DNA vaccines usually are circular pieces of DNA, called plasmids, which contain a foreign gene from a disease agent and a promoter that is used to initiate the expression of the protein from that gene in the target animal (Rodriguez and Whitton 2000). Like recombinant vaccines, genes for the desired antigens are located and cloned. The DNA is injected into the muscle of the animal being vaccinated, usually with a "gene gun" that uses compressed gas to blow the DNA into the muscle cells. DNA can be introduced into tissues by bombarding the skin with DNA-coated gold particles. It is also possible to introduce DNA into nasal tissue in nose drops. Some muscle cells express the pathogen DNA to stimulate the immune system. DNA vaccines have induced both humoral and cellular immunity (Sridhar, 2006).

DNA vaccines can lead to a strong and long-lasting immune response through the inoculation of a plasmid containing a gene for a particular protein antigen, which is subsequently expressed by the cellular machinery of the person receiving the vaccine. DNA vaccines offer the potential for immune therapy of diseases like tumors (Wray and Woodward, 1990). The application of DNA immunization as a new generation vaccine has been well studied since its invention, and a variety of such vaccines have undergone clinical trials, in veterinary practice (Babiuk *et al*., 2007). The DNA vaccines elicit desired immune responses viz. cell mediated immunity (CMI) and humoral immune response (HIR); and it is much easier for their manipulation using recombinant DNA techniques and production in bacteria using fed-batch fermentation (Liu *et al*., 2006).

As an effective vaccine, plasmid DNA has a gene encoding a protective antigen of a pathogen, which when injected into host, is transcribed and translated, to induce a specific immune response. The DNA vaccines, described as genetic immunization to elicit a protective immune response, have been further improved by exploiting various gene delivery methods, cytokine adjuvants and prime-boost (DNA vaccine priming and recombinant protein boosting) approaches (Sharma and Khuller, 2001; Jiang *et al*., 2007). DNA vaccines have several advantages, which include simplicity of manufacture, biological stability, cost effectiveness and safety, ease of transport in lyophilized form and the ability to act in presence of maternal immunity. Besides, different genes can be combined simultaneously, making it possible to develop multivalent vaccines. One of the distinct advantages of the DNA vaccines is the possibility of differentiating infected from the vaccinated animals (DIVA), for effective disease eradication programs. The utility of 'marker' DNA vaccines has been reported for diseases like FMD and avian influenza (Lee *et al*., 2004; Grubman, 2005). The demerits of DNA vaccines, of theoretical levels and not yet proven are integration into host genome, activation of proto-oncogenes, inactivation of tumor suppressor genes and the possibility of generating anti-nuclear antibodies (Sharma and Khuller 2001; Dunham, 2002).

2.3.1.1 Applications of nucleic acid vaccines in veterinary practice

Veterinary vaccinology is a rapidly developing field and currently, vaccines are not only used for the prevention of diseases in animals, but also to help solve public health crisis. Advancement in science and technology, together with improved knowledge in immunology, microbiology and

recombinant technology has played pivotal roles in introducing novel ideas in vaccinology (Liu*et al*., 2006). Shams (2005), has pointed out that subunit vaccines, DNA vaccines and vectored vaccines are rapidly gaining acceptance as new generation animal vaccines. Among many advantages, DNA vaccines also provide DIVA strategy, and hence vaccine-induced herd/flock immunity can be differentiated for effective sero-surveillance (Lee *et al*., 2004).

Bovines' Genetic immunization approach against bacterial diseases of bovines offers attractive possibilities for rapid and effective vaccine development. Against brucellosis, antigen of B. abortus has been utilized for the generation of an effective DNA vaccine (Rivers *et al*., 2006). Against mycobacterial infections in cattle, Huygen (2003) has explained the utility and feasibility of the DNA vaccine approaches. DNA vaccine based on Mycobacterium bovis protein MPB-83 when tested in mice has shown to elicit protective immune responses (Chambers *et al*., 2000). Aside to this, DNA vaccines used in combination with Bacillus Calmette Guerin (BCG) elicited superior protection during challenge studies (Cai *et al*., 2006). Against viral diseases of bovines, the earliest reports suggest the use of a gene encoding the VP4 protein of bovine rotavirus (BRV), found effective in stimulating a Th1-like immune response (Suradhat *et al*., 1997). Also, DNA vaccine encoding the fusion (F) gene of bovine respiratory syncytial virus (BRSV) has been found to induce protection against the infection in calves.

Besides, several workers have developed successful DNA vaccine strategies against bovine herpes virus-1 (BHV-1) infection (Castrucci *et al*., 2005). Nucleic acid vaccine that could protect the cattle against bovine viral diarrhea virus (BVDV) infection has also been developed. VP1 based DNA vaccines are being utilized for developing effective vaccines against foot and mouth disease (FMD) (Dong *et al*., 2005). microparticulate based DNA vaccine has been developed that codes for the T and B cell epitopes of VP1 of the FMDV (Wang *et al*., 2006).

Ovines and caprines Nucleic acid vaccines have been developed that could confer protection tothe common bacterial diseases of sheep and goats. DNA vaccination with genetically detoxified phospholipase D of Corynebacterium pseudo tuberculosis, linked with CTLA-4, protected sheep against caseous lymphadenitis (Chaplin *et al*., 1999). In case of anthrax in sheep, the protective antigen (PA83) gene of Bacillus anthracis has been employed for developing highly promising DNA vaccine (Hahn *et al*., 2006). Para- tuberculosis or Johne's disease, caused by Mycobacterium avium subsp. paratuberculosis, has been successfully controlled by using plasmids coding mycobacterial heat shock protein antigen (HSP-65) (Sechi *et al*., 2006). Against brucellosis, administering DNA vaccine that encode Brucella melitensis outer membrane proteins (OMP), invasion protein B (ialB), periplasmic protein (bp26) and trigger factor (tF) have been found to induce significant immune responses, which could pave way for the effective control of brucellosis in goats (Gupta *et al*., 2007).

Equines: The increasing international movement of horses and the relaxation of regulations have resulted in an increased incidence of equine infectious diseases. The advent of recombinant technology has encouraged the development of new generation vaccines such as live-vectored vaccines and DNA vaccines (Minke *et al*., 2006).

Among bacterial diseases, DNA vaccines have been generated to protect the foals from Rhodococcus equi, which causes pyogranulomatous broncho-pneumonia. Against the equine herpesvirus-1 (EHV-1), a viral pathogen of horses causing respiratory, reproductive and neurological problems, the role of plasmid DNA350 Vet Res Commun (2008) encoding the envelope glycoprotein D (gD) to induce humoral response, has been suggested; and the administration of GM-CSF along with such vaccines significantly enhanced virus neutralizing antibody responses to EHV-1 (Minke *et al*., 2006). For equine influenza (EI), Lunn *et al*. (1999) recorded complete protection of the experimental ponies during challenge after administering DNA vaccine encoding hemagglutinin (HA) gene of EI virus. To prevent the West Nile virus infection in horses, the envelope protein genes (prM and E) have been

incorporated in DNA vaccine formulation to elicit satisfactory protection (Hall and Khromykh, 2004). Similarly, Giese *et al*. (2002) developed effective DNA vaccines encoding the ORF's (5 and 7) of Equine arteritis virus (EAV).

Poultry historically, inactivated whole viruses with various adjuvant systems or live vaccines have been used for the successful prevention of various bacterial and viral diseases of poultry. A plasmid DNA vaccine encoding the enterotoxigenic Escherichia coli K88 fimbrial protein elicited satisfactory protection during E. coli challenge (Cho *et al*., 2004).

Besides, DNA vaccines have been developed against major viral infections of poultry like avian influenza, utilizing the HA gene of the virus (Lee *et al*., 2004). Similarly, a vaccine encoding fusion (F) and haemagglutinin (HN) gene induced higher level of antibodies against Newcastle disease (NDV) in chickens. For Marek's disease, DNA vaccine containing the clone of virulent serotype-1 MDV has been found useful during challenge infection in birds (Loke *et al*., 2005). Vaccination with a mutated, non-oncogenic v-src gene construct, derived from avian leucosis virus (ALV), induced cytotoxic T-lymphocytes (CTL) to protect birds from tumors. Protective utility of plasmid coded N protein gene of infectious bronchitis virus (IBV) has also been reported for which DNA vaccines expressing the S1 glycoprotein of IBV has been suggested (Seo *et al*., 1997). Against infectious bursal disease (IBD), VP2 gene or VP2/4/3 poly-protein gene of IBD virus-based DNA vaccination has been found effective in protection (Li *et al*., 2006).

DNA vaccination against duck hepatitis B virus has been shown to reduce viremia with rapid removal of the virus from the blood after the challenge. Against chicken infectious anemia (CIA), for the first time, the simultaneous in vitro and in vivo expression of viral proteins VP1 and VP2 has been studied for generating protective antibodies against the infection (Senthil Kumar *et al*., 2004). Also, DNA vaccines have been developed against avian reovirus (σC protein gene), and egg drop syndrome (EDS-76) virus (penton fiber gene fragment), and both these vaccines were found effective during their respective challenge studies. Likewise, against protozoan infections, especially coccidiosis, successful DNA vaccines have been developed, utilizing the 3-1E and EtMIC2 genes in combination with cytokines to provide protection from this economically important infection of birds (Ding *et al*., 2005).

Swine Regarding the DNA vaccines developed against swine enzootic pneumonia (SEP), caused by Mycoplasma hypopneumoniae, plasmid DNA coding the heat shock protein gene (P42) should be a suitable candidate as it is capable of inducing both Th1 and Th2 immune responses. Against SEP, the capability of P97 adhesin repeat region of M. hyopneumoniae to produce immunogenicity in mice in DNA vaccine formulation has also been described (Chen *et al*., 2006).

Regarding FMD in swine, DNA vaccines encoding VP1 gene of O, C and A strains of FMD virus showed protection against the disease when administered using 'gene gun' (Benvenisti *et al*., 2001). Researchers have also developed successful DNA vaccines using prM and envelope (E) genes for Japanese encephalitis virus and nucleoprotein (N) gene for transmissible gastroenteritis virus. For constructing DNA vaccines against psuedorabies (Aujesky's disease), the immunogenic viral protein genes such as gB, gC and gD, are often considered (Dory *et al*., 2005). For the prevention of porcine reproductive and respiratory syndrome (PRRS) in swine, DNA immunization strategies could be formulated utilizing the viral ORF 5 region that codes for a major envelope glycoprotein GP5. For endoparasitic infestations like taeniasis (cysticercosis), a DNA vaccine using Taenia solium B antigen has been developed (Guo *et al*., 2007).

Canines During the last couple of decades, immunoprophylactic agents have been developed that have greatly reduced the incidence of infectious diseases of pet animals. Focus has been directed for developing DNA vaccines to eliminate the bacterial diseases like leptospirosis and Lyme disease (Dai *et al*., 2003).Among the viral diseases affecting dogs, the important ones are rabies, canine distemper and parvoviral infections, many studies have been conducted to analyze the utility of DNA vaccines. Initially, it was against the deadly rabies, the first nucleic

acid vaccine was successfully developed (Xiang *et al*., 1994). After this, it was Jiang *et al*. (1998) who reported a plasmid DNA vaccine to protect the canine population from parvovirus infections, utilizing VP1 gene of canine parvovirus (CPV). Later, by using VP2 gene of CPV, a successful DNA vaccine was developed (Gupta *et al*., 2005). The advantage of plasmid-based vaccine against rabies is that it is a valuable alternative for the mass production of cheaper rabies vaccine when compared to the cell culture based ones. A DNA vaccine encoding the rabies virus glycoprotein (G) has been found to yield stronger and more durable virus neutralizing antibody titers in dogs, which has been proven beyond doubt by many researchers (Rai *et al*., 2005).

2.3.2 Recombinant protein (subunit) vaccines

Subunit vaccines contain purified antigens instead of whole organisms. Such a preparation consists of only those antigens that elicit protective immunity. Subunit vaccines are composed of toxoids, subcellular fragments, or surface antigens. Administration of whole organism, as in case of pertussis was found unfavorable immune reactions resulting in severe side effects. The effectiveness of subunit vaccines in increased by giving them in adjuvants. Adjuvants slow antigen release for a more sustained immune stimulation (Sridhar, 2006). Genes from many infectious agents have been cloned and expressed in prokaryotic and eukaryotic cells by using recombinant DNA techniques. Hepatitis B virus (HBV) vaccine is perhaps the most successful vaccine produced so far with these techniques. The current licensed HBV vaccine which contains purified 22 run HB surface antigen, is made from plasma of human carriers and is costly to prepare. Although hepatitis B surface antigen (HBsAG) could be synthesized in E. coli, the yields in this host were poor. However, when the gene was transferred into yeast (*Saccharomyces cerevisiae*), immunogenic particulate forms of HBsAG, which closely resembled those found naturally, were produced and readily extracted (Valenzuela *et al*., 1982). Advantages of subunit are they can safely be given to immunosuppressed people and are less likely to induce side effects. Their demerits are Antigens may not retain their native conformation, so that antibodies produced against the subunit may not recognize the same protein on the pathogen surface and isolated protein does not stimulate the immune system as well as a whole organism vaccine (Sridhar, 2006).

2.3.3 Live genetically modified vaccines

Live genetically modified vaccines could be viruses or bacteria with one or more genes deleted or inactivated, or they can be vaccines carrying a foreign gene from another disease agent, which are referred to as vaccine vectors. Deletion of a gene or genes is to inactivate or attenuate the disease agent. Generally two (double knockout) or more genes are deleted or inactivated so the vaccine remains stable and cannot revert to a pathogenic agent (Uzzau *et al*., 2005). Developing a vaccine of this type requires knowledge of the gene(s) responsible for pathogenicity and assumes that those genes are not the same genes governing viability and the ability of the modified organism to induce an immune response. Examples of gene-deleted vaccines include a *Salmonella* vaccine for sheep and poultry and a pseudorabies virus vaccine for pigs (CAST, 2008).

Another relatively recent method of creating a live genetically modified vaccine is to use an infectious clone of the disease agent. An infectious clone is created by isolating the entire genome of the disease agent (usually viruses) in the laboratory. This isolated or cloned genome can be specifically and purposefully modified in the laboratory and then used to re-create the live genetically modified organism. Vector-based vaccines are bacteria, viruses, or plants carrying a gene from another disease agent that is expressed and then induces an immune response when the host is vaccinated. For viral and bacterial vectors, the vaccine induces a protective response against itself (the vector) as well as the other disease agent. The first commercial vaccine vector was VectorVax FP-N (Zeon Corporation, Japan), a vaccine primarily used in turkeys; it consists of a fowl pox vaccine virus that carries genes from Newcastle disease virus. (CAST, 2008). Other agents used as vectors of foreign genes are *Salmonella*, herpes viruses, adenoviruses, and adeno-associated viruses. Edible plant-derived vaccines take advantage of the ability of some antigens to induce an immune

response when delivered orally. Foreign genes from disease agents have been inserted into potatoes, soybeans, and corn plants and fed to animals; the expressed proteins from those foreign genes immunized the animals against the disease agent (Streatfield 2005).

2.4 Future Developments

Identification and utilization of better immunogens and new vaccines for diseases for which no currently available vaccines exist; Better vaccine delivery methods: oral, intranasal, and needlefree systems allowing mass vaccinations; Use of immunomodulators in vector-based vaccines: CpG motifs and cytokines; Viral vectors to deliver small interfering RNAs (siRNA); Expression of foreign proteins in plants and the development of edible vaccines; Vaccines developed for non-infectious agents: control and prevent cancer and vaccines to induce long lasting contraception (Mark *et al*., 2008).

3. CONCLUSION AND RECOMMENDATION

Vaccines induce an immune response in the animal host that subsequently recognizes infectious agents and helps fight off the disease. It is now possible, through recombinant DNA technology to produce an effective and safer production of both live and killed vaccines with increase response and high specificity. Recombinant DNA technology approach is the identification of that protein component of virus or microbial pathogen which itself can elicit the production of antibodies having capacity to neutralize infectivity, potentially protecting the host against the pathogen. Using recombinant DNA technologies, scientists have been able to develop live genetically modified organisms, recombinant killed vaccines and genetic vaccines that no longer cause disease yet induces a strong immune response. As recommendation when engineered vaccinia virus is used to vaccinate, care must be taken to immunodeficient individuals. Recombinant DNA technology has indeed tremendous breakthrough in the discovery of various vaccines. The new generation vaccines prepared from the viral or microbial proteins; their fragments or nucleic acid sequences have been attractive because of their stability (one of the important characters of a vaccine), non-infectious nature, homogeneity as well as their cost effectiveness. Production of edible vaccines as new generation vaccine is still not as such applicable, it must be widely practiced. This is the proper time for attempting to manipulate genes coding for immunogenic proteins 0f microbes as well as relevant co-stimulatory molecules and develop a vaccine which can elicit mucosal, humoral as well as cellular immune response and provide unequivocal protection to the vaccinated animals.

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