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Biotechnology in the Production of Recombinant Vaccine or Antigen for Animal Health

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Abstract: Recombinant DNA (rDNA) technology has indeed made tremendous breakthrough in the discovery of various vaccines or diagnostic antigens. The advent of rDNA technology and its application in the industry has brought about a rapid growth of biotechnology companies for the production of the rDNA products in human and animal healthcare. The new generation vaccines prepared from the viral/microbial proteins; their fragments or the nucleic acid sequences have been attractive because of their stability, non-infectious nature, homogeneity as well as their cost-effectiveness. Several products are undergoing clinical trials and finally products approval by FDA/FAO/OIE is required before its use in human/animals. Products developed in the field of veterinary prophylactics and diagnostics will be most valuable for further development of rDNA products in the coming decades and advances in biotechnology have also created many legal issues particularly patent. The Biotech industry Guide would be useful for industrial approval, regulatory clearance to rDNA product and biosafety aspects. In view of high market potential for recombinant therapeutics as the case with human therapeutics, indigenous technology should also be developed for the veterinary field to develop a prophylactics and diagnostics. This general article provides collected information regarding production of recombinant proteins and their use in Veterinary science along with brief description of various heterologous expressed systems and their potential applications for the production of recombinant products.

Key words: rDNA technology, production, recombinant vaccine, antigen, veterinary use

INTRODUCTION

Effective control of any infectious disease in an endemic country needs better vaccines along with strong diagnostic support. The difficulty in obtaining large quantities of attenuated/inactivated cell culture viral/microbial antigen is the main hurdle in the production of the vaccines or diagnostic kits. Use of recombinant DNA (rDNA) technology for cloning and subsequent expression of particular gene of interest of virus/ microbes in an appropriate system like bacterial/mammalian/insect/ yeast/plant expression systems will circumvent the difficulties associated with the production of large quantities o f vaccine or diagnostic agents. Such a bio-engineered protein can be obtained in large amounts in a pure and native form. rDNA technology has necessary tools to produce desired viral/bacterial proteins in a native form. Production of antigen in animal tissue culture system using conventional procedure shows batch-to-batch variation, which remains constant in case of expressed protein in any of the heterologous system mentioned above. Recombinant protein can be used as a safe vaccine, a diagnostic antigen or for raising hyperimmune serum. Bulk production of microbial antigens using cell culture needs sophisticated infrastructure, which in turn adds to the cost of production besides involving the risk of handling microbes and release into the environment. The advent of rDNA technology and its application in the industry has brought about a rapid growth of biotechnology companies for the production of the rDNA products in human and animal healthcare. This general article provides collected information regarding production of recombinant proteins and their use in Veterinary science. Article also describes various heterologous expressed systems and their potential applications for the production of recombinant products.

NEW APPROACHES

A safe, potent and cost-effective vaccine is the present day requirement for the effective control of any

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viral disease. As an alternative attempt to avoid the use of live virus, while retaining the myriad of viral antigenic determinants, several works have been carried out for the development of recombinant antigens and new generation vaccines. The emergence of rDNA technology, hybridoma technology, bioinformatics and the more rapid solving of protein structure by X-ray crystallography aided to the major advances which are being used in understanding the host immune response at the molecular level. This knowledge is now available to researcher providing an opportunity to design and develop entirely new products; bearing in mind that protection is directly related to the level of neutralizing antibody in the serum as well as Cell Mediated Immune (CMI) response. The rDNA technology can be used to produce pure antigen (s)/ immunogen (s) for use as diagnostic agent (s)/vaccine(s). Several recombinant proteins produced in prokaryotic as well as in eukaryotic systems have been used as diagnostics and immuno prophylactics.

Traditional vaccination strategies using live attenuated or inactivated viruses have been successful in the past for some of the diseases. However, these strategies may not be appropriate in all the settings, either due to uncontrolled animal movement and extensive crossbreeding for improving the indigenous livestock leading to the evolution of susceptible population or lack of potency of the vaccine. Therefore, it is imperative that newer approaches to develop vaccines are explored at faster pace.

Recombinant antigens and vaccines produced through expression in bacteria, mammalian cells, insect cells and transgenic plants have progressed to a point at which they might offer sufficient advantages over existing, conventional antigens and vaccines in respect of production methodology, biosecurity, stability, antigenic spectrum, speed and duration of the immune response, or cost that would justify the time and investment needed for further development, large scale production, testing and registration. These vaccines showed only partial protection with shorter duration of immunity which may be due to the lack of T-cell epitopes in the expressed protein, improper folding of the peptide or combination of both. Emergence of nucleic acid based vaccines has paved the way for the development of so-called third generation vaccines.

HETEROLOGOUS EXPRESSION SYSTEMS

Prokaryotic (bacteria), eukaryotic (mammalian, yeast) or insects (baculovirus) systems are generally used as a host for the production of usable quantities of the desired rDNA products.

Bacterial expression system: Bacteria such *as E. coli* are widely used for the expression of rDNA products. They offer several advantages viz., high level of recombinant protein expression, rapid cell multiplication and simple media requirement. However, there are some limitations such as intracellular accumulation of heterologous proteins, improper folding of the peptide, lack of post-transcriptional modifications, the potential for product degradation due to trace of protease impurities and production of endotoxin.

Mammalian expression system: Mammalian systems such as Chinese Hamster Ovary (CHO) and Baby Hamster Kidney (BHK) cell systems are the ideal choice for production of therapeutic proteins as these are capable of glycosylating the protein at the correct sites. However, cost of production of the products using these cell systems is high because of the slow growth and expensive nutrient requirement. The choice of an expression system invariably influences the character, quantity and cost of a final product.

Yeast expression system: Among the eukaryotic systems, yeast is unique in that it combines the advantages of both prokaryotic [high expression levels (10-100 fold higher), faster growth, easy maintenance, easy scale-up, inexpensive growth media] and eukaryotic [capacity to carry out most of the post-translational modifications like protein processing, protein folding etc.,] expression systems. Yeast such as *Saccharomyces cerevisiae*, *Hansenulla polymorpha* and *P. pastoris* are among the simplest eukaryotic organisms, which grow relatively quickly and are highly adaptable to large-scale production[1]. These organisms do not produce endotoxin as compared to *E.coli*. Technical advantages in this system include site-specific integration, increase in copy number, leader sequence for the secretion of heterologous protein and post-translational modifications.

In recent past, the methylotrophic yeasts, such as *Hansenulla polymorpha*, *Pichia pastoris* and *Candidia biodini* have been developed, among which *P. pastoris* has emerged as a powerful and inexpensive heterologous system for the production of high levels of functionally active recombinant proteins[2], in addition to existing Saccharomyces. Intact protein production and secretion into the medium makes yeast could be an efficient system for production and purification of the expressed protein.

Insect expression system: Insect cell culture systems are widely used for the production of recombinant proteins, vaccines and viral pesticides as well as in the basic

research in biology[3]. A large number of cell lines from diptera, hemiptera and lepidopteran insects have been established. High levels of heterologous gene expression are often achieved compared to other eukaryotic expression systems, particularly for intracellular proteins. In many cases, the recombinant proteins are soluble and easily recovered from infected cells late in the infection when host proteins synthesis is diminished. Insect cell based systems especially baculovirus based systems revolutionized the recombinant protein production. Baculovirsuses have a restricted host range limited to specific invertebrate species. Being noninfectious to vertebrates, these viruses are safer to work with than most mammalian viruses. Most of the susceptible insect cell lines are not transformed with pathogenic or infectious viruses and can be cared for under minimal containment conditions. Helper cell lines or helper viruses are not needed since the baculovirus genome contains all the genetic information needed for propagation in a variety of cell lines or larvae from different insects. Baculoviruses are usually propagated in insect cell lines derived from the fall armyworm *Spodoptera frugiperda* or from the cabbage looper *Trichoplusia ni*. Commonly used and commercially available insect cell lines are Sf-9, Sf-21 and H-5 (*Trichoplusia ni.*). Prolific cell lines are available which grow well in suspension cultures, permitting the production of recombinant proteins in large-scale bioreactors. The recombinant proteins can be produced in insect cell lines as well as in insects. This recombinant virus can be prepared by cloning any DNA insert coding protein of desire under polyhedron growth promoter or Pro promoter. The recombinant viruses are selected by their inability to induce inclusion body formation 72 hrs after infection of the cell lines or infecting insects for the production of recombinant protein. The production level in this system is very high and if the recombinant virus is prepared for infecting insects then the production of protein is very cheap. However, there is one drawback with this system that glycosylation of protein in insect cells is different from mammalian cells which leads to improper maintenance of epitopes in the target protein.

Transgenic plants: Recent advances have been made in producing therapeutic proteins by using transgenic animates. The plants can be considered as a solar-powered bioreactor and proved to be advantageous over the alternative fermentation systems of biomass production using microbial or animal cells. The requirements for plant system are rather simple and inexpensive. Plants, being eukaryotic, are also capable of the post-translational processing of proteins of eukaryotic origin, which may be essential to their proper functioning. The complex, multi-meric proteins can be readily assembled in plant cells and individual plant expressing genes encoding different components of multi-meric complexes can be readily obtained by sexual crossing of plants harbouring a single transgene. The use of genetically engineered plants to produce valuable proteins is increasing slowly. The system has potential advantages of economy and scalability. However, variations in product yield, contamination with agrochemical and fertilizers, impact of pest and disease and variable cultivation conditions should also be considered. Plant cell culture system combines the advantages of whole plant system as well as animal cell culture[4]. Although no recombinant products have yet been produced commercially using plant cell culture several companies are investigating the commercial feasibility of such a production system.

APPLICATION OF RECOMBINANT PROTEINS

Recombinant proteins are being produced using either of the heterologous systems mentioned above and have a potential value in the development of diagnostic test as well as vaccines for the prophylaxis of various infectious diseases of veterinary importance. A hallmark in the prevention and control of several diseases of the animals and human caused by viruses, bacteria and parasites is the development of suitable vaccines against infectious diseases. So far, various attempts have been made in the recent past to produce antigens in heterologous systems for use as diagnostics as well as prophylactics. Biologically active peptides and proteins have many potential applications including being used as vaccines, immuno-modulators, growth factors, hormones and enzymes. Examples include Infectious Bursal Disease Virus (IBDV) Synthetic peptide antigen based ELISA assay for precise diagnosis[5,6] and anti microbial peptides and peptide hormones for the therapeutics etc. The IBDV peptide-based assay is required for preliminary screening of the field samples to rule out false positive followed by regular diagnostic assay.

DIAGNOSTICS

The E2 protein of Classical Swine Fever Virus (CSFV) has been expressed as secretory protein using the pPIC9K vector in *P. pastoris* and the recombinant protein elicited antibodies against protein E2[7]. Antibody detection method employing Monoclonal Antibody (MAb) raised either against expressed haemagglutinin[8] or nucleoprotein[9] of African lineages of PPR (*peste des petits ruminants*) viruses have been described.

Ismail *et al.*[10] expressed the nucleocapsid gene of virulent Kabete O strain of Rinderpest Virus (RPV) in baculovirus and recombinant protein (rRVN) was used as a coating antigen in an ELISA to distinguish vaccinated animals from those infected with RPV and was also used successfully in the diagnosis of two other morbilliviruses viz., Measles virus and PPR virus. The recombinant N protein of PPR virus produced in insect cells system was used successfully as a coating antigen in an ELISA for the serological diagnosis of PPRV[11]. The success trails on yeast expressed recombinant Tenatus toxoid, Poliovirus, Bovine herpes virus-1, Dengue virus, FMD virus and pseudo rabies viral proteins have been reported[12,-17]. Renukaradhya *et al.*, [18] expressed the recombinant Hemagglutinin (H) of RPV in baculovirus and used for sero-surveillance of rinderpest in MAb based competitive ELISA. The genes coding for the surface glycoproteins Hemagglutinin-Neuraminidase (HN) of PPRV and H of RPV were also expressed transiently in mammalian cells[19] and was found to be biologically active.

PROPHYLACTICS

Sub-unit vaccines: Early observations indicated that isolated immunogenic proteins and their fragments were capable of inducing neutralizing antibodies and conferring partial protection. Till date, various recombinant vaccines produced in different expression systems have been tried with limited success. This may be due to improper folding of the expressed proteins or the difficulties in purification of the protein in native form when expressed inside the cell and the need for other structural proteins to elicit optimum neutralizing antibody response. eg., (i) FMD virus (FMDV) VP1 protein expressed in all heterologous systems (ii) gE-negative pseudorabies virus vector expressing the E2 subunit of Classical Swine Fever Virus (CSFV) and immuno-dominant gE protein expressed in baculovirus as secretory protein and (iii) E2 subunit vaccine for Bovine Viral Diarrhoea Virus (BVDV). The success trails on yeast expressed recombinant FMDV, Poliovirus, bovine herpes virus-1 and dengue virus vaccines have also been reported. Romanos *et al.*[12] produced recombinant *Bordetella pertusis* pertactin (P69) an outer membrane protein in *P. pastoris* with high level of yield and immunological property. Zhu *et al.*[14] expressed truncated BHV-1 gD gene (tgD) in *P. pastoris* as a secretary protein maintaining its native conformation which can serve as a subunit vaccine against BHV-1. The PPR virus F subunit protein and RP virus H protein have been expressed on surface of *Bombyx mori* insect larvae using the baculovirus[20]. Insect cells expressed recombinant H protein of RPV induced humoral and CMI response in cattle[21].

Empty capsid as vaccines: Experimental FMD vaccines have targeted immunogens that contain the entire repertoire of immunogenic sites present on the intact virus, but lack infectious nucleic acid. eg., empty capsids of FMDV retain the antigenicity and immunogenicity of infectious virus particles[22]. Production of empty capsids from whole virus is cumbersome and not practical. However, employing rDNA technology approaches, it has been shown that co-administration of a plasmid encoding porcine GM-CSF together with an FMDV empty construct appeared to improve the FMDV-specific antibody response. Recently, the capsid precursor (P1-2A) proteins of FMDV expressed as a secretory protein in *Pichia pastoris* and the expressed structural protein against type 'O'[16] and other serotypes, Asia1, A_{22} and C was found to induce neutralizing antibodies and protective response, when challenged with FMDV in guinea pigs in a single vaccination[23]. The goat immunized with baculovirus expressed recombinant H-N glycoprotein of PPRV produced immune response against PPRV and antibodies generated in immunized animals could neutralize both PPRV and RPV *in vitro*[24]. These studies and many others indicate the potential of empty capsids.

Synthetic peptide vaccines: Numerous studies carried out with respect to synthetic peptides coding for the immunogenic regions deduced by various methods including monoclonal antibodies lead to the elucidation of T-cell and B-cell epitopes which, in turn, provided a better understanding of humoral and cellular immune responses for most of the viruses including FMDV. The VP1 region of FMDV between amino acid (aa) 21-40 has T-cell epitopes and offers cross protection to serotypes; region between amino acid 141-156 protects homologous virus strains and region between amino acid 200-213 offers cross protection for both homologous and heterologous viruses[25]. In the following years, the concept that the immunogenic peptides should also include viral T-cell epitopes to provide adequate co-operation with immune B-lymphocytes to induce an effective neutralizing antibody response was generally accepted by vaccinologists and a number of such multigene and minigene vaccines were developed. Mice fed with transgenic alfalfa leaf extracts contain expressed FMDV VP1 proteins developed virus-specific immune response to synthetic peptide and to intact virus particle[26]. Biologically active peptides and proteins have many potential applications besides being used as vaccines.

DNA vaccines: In recent years, DNA vaccination (also known as genetic immunization, gene vaccination or nucleic acid vaccination) has emerged as one of the most promising applications of rDNA technology. The potential value of DNA vaccines was realized in 1993 in case of FMDV. Immunization with naked DNA has been reported to elicit humoral and cellular immune response and protection against different pathogens. The concept is based simply on inoculation of the plasmid DNA, which encodes microbial gene(s) under a strong eukaryotic promoter like the early Cytomegalo Virus (CMV) promoter or enhancer or alpha virus. The characteristic advantages of nucleic acid based vaccines over the live attenuated and the whole killed vaccines have widely been appreciated eg. DNA vaccine for Rabies[27,28]. DNA vaccination proves to be a promising venue for recombinant vaccines. The emergence of gene adjuvant has further added a powerful weapon to the arsenal of DNA vaccines.

Cytokine based DNA vaccines: Developments in recombinant vaccine technologies have demonstrated that a single antigen may not always be sufficiently immunogenic against a complex pathogen, necessitating the need to identifying and incorporating several protective antigens in a vaccine construct. Similarly, incorporation of intrinsic immuno-modulatory agents along with vaccine also improves the immunogenicity of a vaccine. DNA vaccine-induced immune responses can be enhanced or modulated by co-administration of the co-stimulatory molecules, pro-inflammatory cytokines, tumor necrosis factors, Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF) and Th1 and Th2 cytokines. The biological activity of cytokines is difficult to maintain under normal conditions and hence their application is limited. However, the rDNA technology facilitates the use of cytokines in vaccination to modulate the immune responses. Inclusion of cytokines and studying their immuno-modulatory effect is a step forward in the development of new generation vaccines. Stimulation of the local pharyngeal immune response can easily be achieved likely by using one of the ever-increasing numbers of identified cytokines, which may be helpful in successful elimination of the virus. These facts made a strong case to develop multigene vaccine constructs wherein multiple genes of important protective antigens and selected cytokine genes, immune (B and T cells) cell receptors like T-cell receptors (TCRs) and immuno-stimulatory sequences like CpG oligodeoxynucleotides (CpG-ODNs) can be cloned in a single plasmid, making it a multigene or multi-component DNA vaccine. Multigene vaccinations can be achieved by either combining different single gene plasmid DNA constructs or by constructing a bicistronic or polycistronic plasmid wherein two or more than two genes are cloned in a single plasmid which expresses multiple genes simultaneously.

Recombinant vector based vaccines: Dangers inherent to the high potential for variation and adaptation exhibited by some viruses like FMDV has hampered the use of classical attenuated strains, obtained by the adaptation and further passages of virulent virus in non-susceptible hosts, as vaccines. This is due to the frequent reversion of attenuated viruses to virulent forms, as well as the fact that viral strains attenuated for a given host may not be attenuated for other natural hosts. The use of adenovirus vectors for delivery of protective immunogens from numerous pathogens is being explored as a vaccine strategy for several diseases. Adenovirus targets the upper respiratory and the gastrointestinal tracts, thereby inducing local mucosal immune responses and triggering cellular immunity. Recombinant replication defective human adenovirus serotype 5 carrying target gene elicited both neutralizing antibody response and protective response when challenged with virulent virus in laboratory animals. eg., FMDV[29]. Capripox recombinant vaccine protected the cattle against RP and Lumpy skin disease[30].

Genetically engineered attenuated (Deletion mutant) vaccine: FMD vaccination is usually discontinued in countries after the disease is eradicated. The stoppage of FMD vaccination is primarily due to the prohibitive cost of vaccination and shorter duration of immunity or protection. However, release of virus from vaccine production plants or the presence of residual live virus in chemically inactivated vaccines is mostly responsible for FMD outbreaks in countries after the stoppage of vaccination during the post-eradication period. This warrants producing safer FMD vaccines through either DNA vaccine approach or genetically engineering the virus to develop attenuated FMD virions. Construction of an infectious cDNA clone has made it easier to directly alter the genetic component of FMD virus. FMD virus genome has been altered by deletion of sequences encoding three amino acid residues, RGD[31]. RGD sequences encode cell binding site and, therefore, RGD deleted FMD virions get attenuated and are unable to spread between normal cells, but can be propagated in cells containing a genetically engineered receptor. Cattle immunized with the RGD deleted mutants did not develop the disease and were protected after FMDV challenge[31].

Marker vaccines: A marker vaccine (live or inactivated vaccine) is either based on deletion mutants or on isolated antigenic proteins that allows the distinction between vaccinated and infected animals on the basis of identifiable difference in antibody responses. A marker vaccine is used in conjunction with a companion diagnostic test that detects antibodies against a protein that is lacking in the vaccine strain[32]. The term marker vaccine is a misnomer because the cardinal feature is not that the vaccine virus differs from the wild-type virus, but that the antibody response of infected animals can be differentiated from that of vaccinated animals[33]. Hence, DIVA (Differentiation of infected from vaccinated animals) vaccines have a negative marker because such vaccines carry at least one antigenic protein less than the corresponding wild-type virus. Besides deletion mutant DIVA vaccines, there are several conventionally-attenuated vaccines as well as inactivated and subunit vaccines that lack the expression of at least one protein in comparison to the virulent virus, thus facilitating in differentiating vaccinated and infected animals. eg., TK deleted mutant Pseudorabies virus DIVA vaccine, gE-negative pseudorabies virus vector expressing the E2 subunit of CSFV and E2 subunit CSFV DIVA vaccine, E2 subunit Bovine Viral Diarrhoea Virus (BVDV) DIVA vaccine. DIVA vaccines for pseudorabies were the first marker vaccines to be developed that could differentiate between infected and vaccinated pigs

Chimaeric vaccines: Adaptation of field isolates as vaccine strains is cumbersome, time consuming and expensive. As an alternative to the adaptation process, the construction of recombinant FMD viruses followed by the production of conventional, inactivated vaccines utilizing these viruses has been proposed. The advantage of such a strategy would be the ability to manipulate the antigenicity of these viruses by substituting the antigenic coding regions (i.e., structural proteins) of a full-length cDNA clone of a suitable strain. Kitson *et al.*[34] constructed poliovirus recombinants containing sequences corresponding to FMDV antigenic sites. Using an infectious cDNA of FMDV, while Rieder *et al.*[35] constructed FMD type A viruses in which the βG-βH loop had been substituted with the homologous sequences from serotype O or C. Though these vaccines were safe theoretically, they have their own potential disadvantages, which include chances of recombination leading to production of virulent viruses *in-vivo*. Chimaeric viruses in which the RGD receptor-binding site or the L gene was deleted induced protection in natural host without producing clinical symptoms. In general, the chimaera was found to be thermally less stable than the parental strain, suggesting it to be an inferior strain for inactivated vaccine production. In spite of these promising results, the wide FMDV host range and the high potential for variation of the virus makes a careful study of the stability and pathogenicity of new recombinant vaccines necessary before they can be considered for field trials.

Self-replicating genetic vaccines: A major rationale for putting antigen-coding genes under the control of alpha viral RNA replicase was to enhance antigen expression and presentation. A fundamental difference between replicase-based DNA vaccines and conventional DNA vaccines is that the virus-like RNA replicates inside the transfected host. Transfection of host cells with replicase-based genetic vaccines could trigger a series of danger signals[36]. Replicase-based DNA or RNA induces apoptotic death of the host cell *in-vitro* just as alpha viral infection and these apoptotic cells may be picked up by dendritic cells for presentation to the immune system. Transfection with self-replicating genetic vaccines may also cause the production of heat shock proteins, in transfected or by-stander cells[37]. The activity of the viral replicase may provide a powerful adjuvant effect because of the requisite production of double-stranded (ds) RNA intermediates. dsRNA itself is a potent inducer of the IFN and virus derived ds RNA can function as a strong adjuvant for cellular and humoral immune responses. Several molecules are known to bind to and can be activated by dsRNA. The best characterized are 2'-5' oligo adenylate (2-5A) synthetase and activated protein kinase-RNA (PKR). The cellular death observed in response to dsRNA is likely to be mediated by both the 2-5A system-induced RNase as well as substrates of PKR. IFN-gamma potentiates the apoptotic effects of dsRNA[38]. Thus Sindbis virus self-replicating RNA vectors have high potentiality for being considered as media for the development of new generation vaccines for many viral diseases including Cancer[39,40].

Edible vaccines: Oral vaccination is considered to be an alternative ideal approach as several recombinant vaccines have proved to be efficacious. Vaccinia-recombinant rabies vaccine as well as crude and purified glycoprotein, when ingested/administered orally, have proven to be effective inducing protective immunity in wildlife[41]. In this context, the concept of vaccine production in transgenic plants assumes significance because of the relatively low-cost due to crop-based production system, which does not require the sophisticated and expensive cell culture and fermentation-based vaccine production plants. The overall cost of

production, relative to the economics of vaccine production in alternative systems, is the main determinant of the utility of crop plants as an expression system for bio-pharming. Transgenic plants expressing vaccine antigens in their edible tissue has proved to be an inexpensive oral vaccine production and delivery system, making the immunization possible simply by consumption of edible vaccine[42]. Stable expression of transgenes into the plants offers advantages of subsequent generation of a large number of transgenic plants, either by vegetative or by sexual means and the opportunity to construct transgenic plants for multi-component vaccine production. Additionally, the selection of proper regulatory elements allows organ-specific or tissue-specific expression of foreign genes. A number of plant-based vaccines against viral, bacterial and other diseases have been devised employing various expression strategies and are in various stages of development and trials. Expression of antigens in transgenic plants has been increasingly used as alternative to classical methodologies for antigen expression for the development of experimental vaccines. eg., Foliar extracts of the plants from a Tobacco mosaic virus-based vector carrying complete FMDV VP1 protein showed specific antibody response against VP1 as well as whole virus and elicited a protective response[43]. Also, these results demonstrated the possibility of using a novel and simple methodology for obtaining transgenic plants expressing high levels of foreign proteins, which could be directly applied in the development of plant based vaccines for veterinary use. In developing countries, efforts at mass immunization of animals and human beings against many diseases are hampered by the relatively high cost of vaccine production. McGarvey *et al.*[44] expressed the rabies virus glycoprotein in transgenic tomatoes, for its potential immunogenicity. Transgenic plants (tobacco, peanut) expressing the H protein of RPV produced specific immune response in Mice and cattle[45,46].

In India, therapeutics like insulin, FSH, GH, GM-CSF, G-CSF, factor VIII, IFN-alfa and gamma, IL are already approved for human use [47]. Indian companies either through foreign collaboration or through total indigenous efforts successfully developed these products. In India, all the recombinant products are considered to be new according to the current Indian Drug Act, 1988 and therefore require the permission of Drug control Authorities for both import and local production for marketing purposes. The future of rDNA products as a human therapeutic looks very bright[48]. The recent advances in biotechnology have created many legal issues, particularly under the patent system. In future, we hope that, the veterinary rDNA products also look bright for the field condition with respect to diagnostics and prophylactics.

CONCLUSION

rDNA technology has indeed made tremendous breakthrough in the discovery of various vaccines or diagnostic antigens. The new generation vaccines prepared from the viral/microbial proteins; their fragments or the 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. This is the proper time for attempting to manipulate genes coding for immunogenic proteins of 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. Several products are undergoing clinical trials and finally products approval by FDA/FAO/OIE is required before its use in human /animals. Products developed in the field of veterinary prophylactics and diagnostics will be most valuable for further development of rDNA products in the coming decades. Recent advances in biotechnology have created many legal issues, particularly under the patent system. The discovery and initial characterization of any rDNA product of potential therapeutics application are followed by its patenting. The Biotech industry Guide would be useful for industrial approval, regulatory clearance to rDNA product and biosafety aspects. It would also provide help to scientists in obtaining patents on the inventions in India. In view of high market potential for recombinant therapeutics as the case with human therapeutics, indigenous technology should also be developed for the veterinary field to develop a prophylactics and diagnostics. This can be achieved by strengthening the linkages among various institutes having expertise in different disciplines related to rDNA technology and increased interaction with the industry.

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