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Applications of genetic engineering in veterinary medicine

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Abstract

A mutation of just one gene will cause abnormal cell behavior leading to the synthesis of a dysfunctional protein. This mutation will inevitably result in the cell functioning only marginally or not at all. Other genetic mutations interfere with the cell's normal life cycle, especially the cell-division cycle. The goal behind recombinant DNA technology is to deliver the correct version of a mutated gene to the cell so that the expression will lead to the normal production of protein and the restoration of normal cell function. This can be considered qualitatively different from other conventional treatments due to genetic material being a putative therapeutic agent. By altering the genetic material of cells, gene therapy may correct, or one day cure, the specific disease pathophysiology. Genetic engineering has been used in veterinary medicine to diagnose, prevent and treat diseases, breed different species and produce transgenic animals for therapeutic proteins or xenografting. In this review the current status of recombinant DNA technology and its application in veterinary medicine together with the obstacles to, and applications of, genetic engineering in veterinary medicine are discussed. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Recombinant DNA; Gene therapy; Genetic engineering; Transgenic animals

Contents

1. Introduction

There are many obstacles to overcome in gene therapy, including cell and nucleus entry, intracellular stability of vectors, transfection and ethical issues. Difficulties at the basic level include shortcomings in gene transfer vectors and an inadequate understanding of the biological interactions of these vectors with the host [1–3].

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Successful gene therapy not only requires the identification of the appropriate therapeutic gene but also relies heavily on the delivery system in which the gene can be delivered both efficiently and accurately [3]. The delivery system must be restricted in such a way as to leave the normal cells unaffected by any detrimental affects of bystander cell transduction. Targeting may be achieved by engineering the surface components of viruses and liposomes at the level of target cell recognition and/or by incorporating transcriptional elements in plasmid or viral genome [4]. In the enthusiasm to proceed to clinical trials, basic studies of disease pathophysiology have not been given adequate attention. However, basic studies are essential since, when they are carried out in appropriate animal models, they can lead to alternative treatment strategies, a better understanding of target cells and an overall more effective design of a therapeutic approach. Studying the differences between human diseases and animal model phenotypes may lead into insights of disease pathogenesis that may be exploited either by gene therapy or pharmacological approaches. As additional genes leading to human diseases are isolated, gene targeting and transgenic technologies will generate more mouse models of human diseases and it is anticipated that an increasingly productive use of such models to elucidate disease pathophysiology will lead to more efficient gene therapy approaches.

2. Veterinary applications

2.1. Diagnostics

The number of applications of nucleic acid probes and DNA products such as monoclonal antibodies for clinical and diagnostic use in veterinary medicine are increasing rapidly.

2.1.1. Nucleic acids

The polymerase chain reaction (PCR) is having a major impact in the diagnostics area of veterinary medicine. The broad applicability of PCR is due to the sensitivity that permits enzymatic amplification of gene fragments from minute quantities of nucleic acids derived from limited amounts of material. Aside from the diagnosis of infections, the PCR can be utilized in identifying parasites and their genetic characterization, the isolation and characterization of expressed genes and the detection of anthelmintic resistance [5]. The PCR is so sensitive that just a single virus, bacterium, parasite or cell is sufficient to be detected, provided part of its nucleic acid sequence is known. Since the nucleic acids are much more stable than enzymes or other proteins, the material does not need to be fresh or stored under special conditions. The PCR also tends to be quicker than immunodetection methods and has been standardized so it can be carried out in diagnostic laboratory [6].

Much research has been preformed in the study of feline infectious peritonitis (FIP). With a common subset of neurological disease, FIP is a fatal Arthus-type immune response of cats and may occur systemically or in any single organ system. Antemortem indicators of this disease are a positive anti-coronavirus IgG titer in cerebrospinal fluid, a high serum protein concentration. Findings using magnetic resonance imaging suggest periventricular contrast enhancement, ventricular dilatation, and hydrocephalus. Postmortem diagnosis may be detected using a technique of FIP monoclonal antibody staining of the affected tissue with a coronavirus-specific PCR [7].

Viremia with feline coronavirus does not necessarily lead to FIP and death, thus making a positive feline coronavirus of low prognostic and diagnostic value. However, the utility of a feline coronavirus-specific PCR may be more reliable in samples of peripheral blood mononuclear cells (PBMCs) and such a test can be used to further study the pathogenesis and epidemiology of FIP [8]. In a parallel study, a dot blot hybridization assay was found to be of value in coronavirus infected cats. Using a biotinylated cDNA probe the assay was able to detect FIPV RNA, in particular cells infected with FIPV isolates. In an in vivo study, the probe was used to detect FIPV RNA in peripheral blood mononuclear leukocytes (PBML) from cats infected with an FIP-producing coronavirus isolate [9].

A reverse transcription, semiquantitative PCR can detect changes in PBMC cytokine mRNA levels by assessing specific interleukins (ILs) and an interferon

(IFN). This study was preformed using two recombinant FIPV (feline infectious peritonitis virus) spike proteins into 8-week-old kittens, which were challenged with FIPV 79-1146. All of the kittens developed signs of FIP and where then confirmed on a gross post mortem examination. The recombinant proteins induced no specific antibody response and failed to alter the course of the disease, but a week after the virus challenge the PBMCs showed small increases in the expression of a IL-6 and IFN gamma mRNA. It was also noticed that as FIP developed, mRNA levels of various IL's and IFN gamma became markedly depressed [10].

A PCR assay developed to detect bovine retrospumavirus (bovine syncytial virus; BSV) used two different sets of oligonucleotide primers. These primers were used and compared for their ability to amplify the targeted BSV sequences by PCR. The specificity of the PCR for both primer sets tested was confirmed when the amplified cDNA products of expected size reacted positively with the corresponding virus-specific cDNA probes in specific assays. By targeting specific gene regions of the BSV provirus genome, the PCR can be used for the confirmation of the presence of BSV in cell cultures used for virus isolation or for the diagnosis of infection in bovine PBL's [11]. Abed et al. [12] found that it was possible to detect a bovine-immunodeficiency-like virus by using capsid and transmembrane envelope proteins from recombinant baculovirus. A positive detection of the presence of anti-BIV antibodies confirmed the use of recombinant proteins as test antigens for serodetection of BIV-infection in animals [12].

The polymerase chain reaction can also be utilized for identification of food spoilage microorganisms. The PCR along with other DNA based typing, most notably fingerprinting methods, enables a clear insight in the identity of microorganisms on different levels varying from genus to strain level. By discriminating between subspecies and strain level it is possible to investigate the route and source of contamination [13].

2.1.2. Monoclonal antibodies

The application of immunological techniques has been responsible for the exponential growth in understanding biochemical research, biochemistry and genetics. The impact of monoclonal antibodies (MAbs) on diagnostic technologies and therapeutic regimes has been equally dramatic but is limited by the manner in which antigen-antibody interactions can be controlled. This use of MAbs is also hindered by the ability to consistently produce antibodies with appropriate affinity and specificity [14].

Biotechnological methods continue to show promise for improved diagnostic and prophylactic purposes. Production of MAbs directed against viral and bacteria-specific antigens such as, classical swine fever virus, bovine virus, diarrhea virus, animal parvoviruses, feline leukaemia virus, Alphavirus, Brucella and Francisella all are currently being used in some biotechnological techniques [13].

A variety of MAbs are highly specific and effective instruments in studying the hog cholera virus. By using these MAbs to study the viral antigenic structure and functional characteristics of surface proteins it became possible to single out and map the functions of antigenic sites of surface glycoprotein E2. Mapping out these functions led to the detection of the relationship between RNAse activity and the structural component of another surface glycoprotein, E0 [15]. As it turns out the envelope glycoprotein E2 is the most immunogenic protein of classical swine fever virus (CSFV). The immune response induced by E2 protects pigs against CSFV and an indirect blocking ELISA, or a complex blocking assay (CTB) based on two independent structural antigenic units is routinely used worldwide for serological diagnosis of CSFV infections [16]. Studying MAbs by measuring the phenotypic changes in circulating leukocytes can also be implemented as a diagnosis indicator in CSFV infection. The pattern of phenotypic change varied with the virulence of CSFV. Infection with virulent strains resulted in the dramatic early loss of CD8-bearing T lymphocytes from the circulation [17].

The study of the application of ELISA (Enzyme-Linked Immunosorbent Assay) to detect antibodies in the serum of sheep against Dichelobacter nodosus assisted the confirmation of the potential of ELISA in veterinary medicine. By measuring the antibodies against D. nodosus using ELISA it was possible to detect the recent occurrence of virulent footrot in young sheep, which in turn could be utilized in the inappropriate use of vaccines in footrot protected

areas and to confirm prior vaccination of sheep. Aside from assessing the vaccination status of flocks, this test could prove useful in farms where the virulent footrot has been eradicated from breeding stock and where confirmation of freedom from active infection is sought in young sheep after a period favorable for transmission [18].

With the use of two MAbs a simple, reliable and rapid blocking ELISA ('Ceditest') can detect Bovine Virus Diarrhoea Virus (BVDV) specific antibodies in cattle serum, plasma and bulk milk. The blocking ELISA is able to detect specific antibodies in serum obtained 12 days after an acute infection and in serum of vaccinated and challenged animals. This specific ELISA test can be preformed with high reproducibility using undiluted samples, and can reliably be used as diagnosis of BVDV infections in large-scale screening and eradication programs [19].

2.2. Disease prevention and treatment

Immunological veterinary medicine, such as veterinary vaccines, contribute to the health of animals, reduced economic losses resulting from diseases, and both increased agricultural productivity and assurance of high quality food for the customer. DNA vaccines and their application in veterinary medicine have been explained in a recent paper [20] and in this Theme Issue by the other authors. The types of diseases under consideration for somatic gene therapy are diverse and have many different underlying causes. Therefore, the rationales and strategies for treating particular diseases are varied.

Many inherited diseases result from mutation of a single gene such as sickle cell anemia, hemophilia's, cystic fibrosis, and hypercoholesterolemia. Hemophilia A and hemophilia B are both X chromosome-linked recessive bleeding disorders and results from a deficiency in the coagulation factors VIII and IX, respectively. DNA technology has shown success, demonstrating partial persistent correction in canine models of hemophilia A and B [21]. Gene therapy for hemophilia A has since reached the same developmental stage as that for hemophilia B. It has been established that reduced expression of human clotting factor VIII cDNA is caused by transcriptional regression. To increase the expression of factor VIII improved Retroviral vectors and adenovirus-based vectors have been developed. The use of such vectors has resulted in clinically relevant levels of human factor VIII in mice and hemophilic dogs [22].

The polymerase chain reaction (discussed earlier) was used to amplify the entire coding region of canine factor IX from a hemophilia B animal. The mutation responsible for canine hemophilia B resulted in a complete lack of circulating factor IX in the affected animals. The observed mutation would have major adverse effects on the tertiary structure of the aberrant factor IX molecule. The elucidation of this mutation sheds light on structure-function relationship in factor IX and should facilitate future experiments directed toward gene therapy in this disease [23]. In a similar study, recombinant adenoassociated viral vectors (rAAV) containing the canine factor IX cDNA was infused into the livers of canine models of hemophilia B. When a dose of rAAV was administered to hemophilic dogs, results showed 1% of dogs with normal canine factor IX levels, the absence of inhibitors, and a sustained partial correction of the coagulation defect for at least 8 months [24].

Herzog et al. [25] used a viral gene delivery system in which a series of percutaneous intramuscular injections of an adeno-associated viral vector was administered at a single time point with no association with local or systemic toxicity. Five hemophilia B dogs were treated and showed stable, vector dose-dependent partial correction of the whole blood clotting time and, at higher doses, of the activated partial thromboplastin time [25].

Krabbe disease is a severe disorder of the peripheral and central nervous system myelin caused by deficient galactocerebrosidase activity (GALC). This auotosomal recessive disease affects humans and animals including dogs, mice and rhesus monkeys. Baskin et al. described the clinical, pathologic and biochemical features of the affected rhesus monkey [26]. Affected monkeys had very low GALC activity and a two base pair deletion in both copies of the GALC gene. Clinical signs of tremor, hypertonia and incoordination led to humane euthanasia by 5 months of age. At neuroscopy, peripheral nerves were enlarged. The results revealed that rhesus monkey model can be useful for exploring treatment options, including prenatal bone mar-

row transplantation and various approaches to gene therapy [26].

With common diseases, such as, coronary heart diseases and diabetus, typically several genes are involved thus making a single gene mechanism exceptional. Knowledge of pathophysiology is beginning to suggest how in particular instances the introduction of specific genes might reverse or retard disease processes at the cellular level. This general approach may prove effective regardless of genetic etiology and without the need to replace a single, missing gene product.

Over the past two decades, studies have revealed cancer as a genetic disease at the cellular level and a multistage process driven by inherited and somatic mutation of cellular genes, followed by clonal selection of variant cells with increasing aggressive growth properties. Cytokines may be used effectively when administered systemically and may offer substantial clinical benefit in certain cancers and viral infections. Such products may have significant clinical impact on highly fatal disorders of canine, however these products when administered systemically perturb complex regulatory pathways resulting in serious side effects [27].

Melanoma is a common neoplastic disease of dogs with varied presentation and biological behavior. Canine malignant melanoma is a rapidly metastatic disease that is, in general, incurable. Standard therapeutic approaches such as chemotherapy and radiotherapy have yet to have been proven effective in canine malignant melanoma. A recent genetic therapy study has shown promise in reducing the mortality of dogs affected with malignant melanoma [28]. In another study, the antigen identified by MAb's was observed to be a well-conserved, highly expressed cell surface protein that was present during all phases of the cell cycle during malignant canine melanoma. This consistency in expression suggested that the antigen may be potentially useful to treat dogs with melanoma [29]. It was found that by treating metastatic melanoma in canine with tumor surgery, radiotherapy, and repeated local injections of xenogenic Vero cells relapse less frequently and survive longer than those dogs treated with surgery and radiotherapy alone. Xenogenic Vero cells were observed to secrete high levels of human IL-2, and be produced local to the tumor site which promoted a strong immune response leading to tumor growth inhibition or rejection [30].

The safety and efficacy of intratumoral injections of a bacterial superantigen with a cytokine gene in dogs with malignant melanoma was evaluated by Dow et al. [31]. The dogs with melanoma were treated with lipid-plasmid DNA encoding staphylococcal enterotoxin B and either GM-CSF or IL-2. Results showed that survival times for animal with stage III melanomas treated by intratumoral gene therapy were prolonged significantly compared with animals treated with surgical excision alone. In addition, local tumor transfection with superantigen and cytokine genes were capable of inducing both local and systemic antitumor immunity in an outbreed animal with a spontaneously developing malignant tumor [31].

Newly hatched chickens are highly susceptible to infection during the first two weeks of life. The utilization of cytokines as therapeutic agents in livestock animals, in particular poultry, has become more feasible with the recent cloning of cytokine genes and the progression of new technologies, such as, live vectors. Lowenthall et al. [32] constructed a live recombinant fowlpox virus (FPV) that expresses chicken myelomonocytic growth factor (MGF). Administration of MGF to chicks resulted in a marked and sustained increase in the number of circulating blood monocytes as well as an increase in their state of activation, as measured by enhanced phagocytic activity and elevated production of nitric oxide. Administration of IFN improved chick's resistance to disease challenge [32].

Cytokine gene engineered tumor vaccines are currently an area of intense investigation in both basic research and clinical medicine. Hogge et al. [33] utilized tumor vaccines in an immunotherapeutic manner involving canines with spontaneous tumors. The dogs were vaccinated with primary auotologous tumor cells transfected with hGM-CSF or a reporter control gene. Exogenous protein was detected at 24 h post injection and dramatic histological changes were observed, characterized by neutrophil and macrophage infiltration at the sites of injection of GM-CSF transfected cells [33].

Although there are only a few well-characterized primary immune deficiency's (PID) of food animals, these diseases are important because they tend to be

severe and have no cure. Most animals with PID do not receive the intensive and aggressive care required for survival. Therefore, veterinarians should be aware of these disorders and seek a correct diagnosis [34].

2.3. Animal breeding

For a long time, human beings have been breeding animals to increase their strength, feed efficiency and disease resistance. Recent advances in molecular biology have created the belief that animal breeding can be revolutionized in future. The quantitative trait locus approach (QTL) (typing DNA markers in segregating populations) has been successful in identifying many chromosomal regions that are involved in growth, body composition and reproduction in animals. Potential QTL influencing traits, such as fat thickness, retail product yield, ovulation rate, meat tenderness and dairy form have been identified in beef cattle. Therefore, the use of new genomic tools to study gene expression changes will prove powerful in the dissection of selection response into its underlying components (QTL) [35].

Quantitative trait loci (QTL) affecting milk protein percent were mapped in the Israel Holstein dairy heard, using selective DNA pooling on a sample date base comprising 2300 milk samples collected from the daughters of seven sires. In the second stage 6500 milk samples were collected from the daughters of 10 sirens. These daughters comprised the high and low phenotypic extremes for protein percent and for milk yield and protein yield. Seven markers on six chromosomes showed significant effects on protein yield and 12 markers on nine chromosomes showed significant effects on milk yield [36].

In another study, Rapacz et al. [37] showed the effect of 25% barley and 2% evening primrose on lowering cholesterol in egg yolk and also variations in the profile of fatty acids in yolk from 40 hens derived from two commercial crosses: white shell eggs and brown shell eggs. Results revealed that, the use of genetic markers in association with environment could be used as a tool for the production of eggs for human consumption with a more desirable content of fatty acid and cholesterol [37]). Loss of function mutations in the myostatin gene (MSTN) produce muscle hyperplasia in cattle termed ‘Muscular hypertrophy’, and in the mouse ‘Compact’. Lord et al. [38] determined whether comparable mutations were present in this gene in meat breeds of sheep and cattle. Preliminary data suggested that MSTN mutations caused loss of function in cattle and different mutations may affect its expression and hence muscularity [38].

Genes, that encode for cytokines are potential candidates for disease resistance quantitative trait loci in cattle. Relatively few members of this gene family have been positioned on the bovine genetic map. Informative markers associated with cytokine genes may be incorporated into a genome wide QTL scan of a resource cattle population with defined levels of heritable resistance to parasitic nematode infection [39].

2.4. Transgenic animals

With some exceptions the process of producing transgenic animals has not been straightforward and various manipulations have been performed, many of which are still at a fairly early stage of development. Human growth hormone was introduced in mice and pigs in early experiments, but many problems were found, thus halting future studies. So far, the largest of these has been in producing transgenic mice to model human diseases. There are sufficient similarities between human and mouse genes, once a human gene has been identified the equivalent gene in a mouse can disable to observe similar effect. Alternatively, an oncomouse has been produced to contain a genetic defect. This caused controversy when the mouse became the subject of a patent application. A generally less controversial approach has been the novel idea of genetically engineered mammals to produce proteins in their milk to serve potential medical benefit as pharmaceutical agents [40]. The leading examples in the area are shown in Table 1.

Another potential use of genetic engineering is tissue or organ transplants. Xenografting offers the potential to use animal organs as transplants into humans, such as hearts and kidneys. Scientists hope that genetically engineered animals with human genes can produce a therapeutic protein and send the correct signal to the human. However, there are many problems that need to be addressed, including rejection of transplant tissue. Moreover, as pointed out at the time of the earlier ‘Dolly’ announcement,

Table 1
Transgenic proteins in clinical trails

Product	Disease	Status
Alpha1 antitrypsin	Cystic Fibrosis	Phase II
Antithrombin	Coronary	Phase III
Collagen	Arthritis	Preclinical
Superoxide dismutase	Respiratory	Preclinical
Salmon calcitonin	Osteoporosis	Preclinical
Protein C	Vein	Preclinical
Factor VIII	Hemophilia A	Preclinical
Factor IX	Hemophilia B	Preclinical
Lactoferrin	GI infection	Preclinical

much development work is necessary and particular aspects will need ethical acceptance.

3. Conclusions

This review has considered only some of the recent progress that has been made in genetic engineering and its application in veterinary medicine. There appears to be a clear and legitimate need to address the major difficulties associated with this area of research, including safety and ethical issues, before proceeding to any application of the research findings.

The review has highlighted that it is important to define some fundamental principles in animal biotechnology such as beneficence, non-moleficence, justice and respect for the integrity of the animal.

We conclude that despite the significant problems and challenges associated with this area, recombinant DNA technology has the potential to revolutionize the vaccination of animals, promote the production of recombinant proteins to treat deadly diseases in both animals and humans and also has potential for nutritional applications. Moreover, this technology could help the scientist to develop accurate diagnostic tests for common animal diseases thereby increasing overall animal efficiency while aiding farmers economically.

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