Biotechnology and the Bovine Practitioner

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Introduction

Biotechnology has been described as one of three major breakthroughs for agriculture: diesel power in the 1920's, the chemical era of the 1950's, and biotechnology in the 1980's. By definition, biotechnology is "the use of cellular elements to produce a useful product or effect." This, in itself, is nothing new. What is new is the explosion of new technology that has occurred in the last 10 to 15 years, allowing us to dissect out and recombine cellular elements at the DNA level. As our ability to understand and utilize cellular elements increases, product usefulness increases exponentially. For example, one new technique that is developed may result in hundreds of new products. However, when two techniques can be combined to work synergistically, the result is not two hundred products, but thousands of new products. One example of this would be the combined use of monoclonal antibody techniques and recombinant DNA techniques for subunit vaccine production.

The two most widely applied biotechnological advances are recombinant DNA technology (genetic engineering, "gene splicing") and monoclonal antibody technology. Recombinant DNA technologies have been applied to the organic synthesis of proteins (insulin, interferon, interleukin-2, etc.), production of subunit vaccines, insertion of genes into non-pathogenic viruses, and deletion of virulence genes from pathogenic viruses. Monoclonal antibodies have revolutionized the diagnostics industry by creating a constant source of antibodies for diagnostic test kits such as the enzyme-linked immunosorbant assay (ELISA), precluding the necessity for constant use of goats, rabbits, and horses for hyperimmune serum production. They have also provided a source of antibodies for passive transfer of specific antibodies to neonates and have been widely investigated in human medicine for potential therapeutic use in cancer patients.

The basic concepts of many new biotechnological advances are relatively simple to understand. Unfortunately, the application of these techniques in developing new products is extremely tedious, complex, time-consuming, and expensive. In this paper, the basic concepts of some new biotechnology techniques are discussed, with examples given of present and future potential uses for veterinary medicine.

Recombinant DNA Technology

Production of Synthetic Proteins

The basic principle of recombinant DNA technology is the isolation of segments of DNA called genes. The genes are contained in double-stranded helical molecules of DNA. Within each gene are triplets of base pairs called "codewords" that code for the production of specific amino acids. Since proteins are made up of amino acids, many such codewords are present in a gene that codes for the production of s specific protein. If the desired synthetic protein is human interferon the gene which codes for interferon production is isolated and inserted (recombined) into the DNA of another organism such as *E. coli* or yeast. The classical scheme involves recombination into plasmids of *E. coli* bacteria. Plasmids are circular pieces of DNA that can be transferred from one bacterium to another. Thus, *E. coli,* which can readily be grown in large bacterial cultures, is effectively being "taught" to produce a foreign protein. Recombinant DNA techniques for synthesizing proteins have several advantages over traditional methods. They can be used to efficiently and economically produce proteins in relatively pure form. One example is human recombinant insulin, which is now available for treatment of diabetes. Because the human insulin gene was used in production, the problem of allergic reactions to bovine and porcine insulin in diabetics is also alleviated. Synthetic proteins of veterinary importance which have been produced by recombinant techniques include bovine interferon and bovine interleukin-2.

Production of Subunit Vaccines

Most of the vaccines and bacterins we presently use in both human and veterinary medicine are either modified live whole organisms or killed whole organisms. Subunit vaccines are those produced using only a piece of the infectious agent. For example, the exact piece of a virus which is responsible for stimulating neutralizing antibody production can be isolated and used for immunization. The recombinant DNA techniques described above are used to synthesize these antigenic proteins. Monoclonal antibodies, which are discussed below, are an invaluable screening tool when searching for the genes that produce the exact protein

desired. Subunit vaccines have several advantages over modified line vaccines: There is no risk of viral mutation, infection of herdmates, and vaccine-derived illness. When produced recombinantly, the subunit antigen can be produced in large amounts and in a relatively pure form. Recombinant subunit viral vaccines have been produced for human hepatitis B virus and influenza, among others. At present, these techniques represent one of the best hopes for developing an effective vaccine for AIDS. Since some of the non-antigenic proteins of the AIDS virus are immunosuppressive, a killed whole-virus vaccine would be counterproductive. In veterinary medicine, a recombinantly-derived subunit vaccine for foot and mouth disease has been produced. Subunit vaccines can also be produced by conventional, non-recombinant techniques. The feline leukemia virus vaccine presently being used is an example of such a subunit vaccine. The possibilities for future recombinantly-derived subunit viral vaccines in bovine medicine are limitless. Some which may be available in the next 5 to 15 years include IBR of cattle, non-cytopathic BVD, and bovine leukemia virus, among others.

Insertion and Deletion of Viral Genes

One technique that may hold great promise is the insertion of desirable genes from pathogenic viruses into a non-pathogenic laboratory strain of vaccinia virus. This technique has the same advantage as subunit vaccines in that the exact portion of the pathogenic virus that is responsible for stimulating antibody production can be spliced into the vaccinia virus vector. It has further advantages in that the vaccinia virus itself is still able to replicate in the host, thereby stimulating cellular immunity as well as humoral immunity. Numerous vaccinia-derived human vaccines have been developed that appear to be highly effective and safe.

Another technique which holds promise is that of deleting the gene responsible for infectivity of a virus. For example, the gene responsible for producing an enzyme that the virus needs to reproduce can be deleted. One example of this technique is a vaccine that was recently developed for pseudorabies in swine.

Although both of these types of vaccines appear to be highly effective, public acceptance of them may hinder future development efforts. With conventional techniques, a virus is grown and allowed to replicate multiple times in cell culture until some *unknown* mutaton occurs which makes the virus non-pathogenic. It can be argued that recombinantly-derived subunit vaccines are also modified live vaccines, the only difference being that we now **know** what the mutation is. However, fears about releasing genetically engineered organisms into the environment still exist.

Monoclonal Antibody Techniques

A monoclonal antibody is an antibody produced by a clone (populations of identical cells) of antigen-stimulated B-cells. If an antibody is desired to a particular antigen, the antigen is first injected into a mouse. This stimulates the mouse's B lymphocytes to produce specific antibodies to the antigen. The mouse is sacrificed and its spleen cells are fused with tumor cells called myeloma cels. Since there are many B- lymphocytes in the spleen cell population, the hope is that some of the ones making antibody to the antigen will fuse with the myeloma cells, and virtually "teach" the myeloma cells to produce the specific antibody. The cells producing the desired antibody must then be isolated and grown up in a pure population. Since myeloma cells grow continuously (indefinitely) the end result is a population of tumor cells that grow indefinitely and produce pure antibody to a particular antigen. In human medicine, monoclonal antibodies have been developed against specific tumor antigens for potential use in cancer therapy. Another anti-cancer use has been linking these antibodies to chemotherapeutic agents so that the toxic substance is concentrated in the body at the tumor site. In bovine medicine, a monoclonal antibody to the K-99 pilus antigen of *E. coli* has been developed for oral use in passive transfer of immunity to neonatal calves. In addition, several monoclonal antibodies have been developed for use in diagnostic testing of veterinary infectious diseases. One is the monoclonal to the K-99 antigen for use in detecting colibacillosis in calves. Monoclonal antibodies have also been developed for ELISA testing for feline leukemia. This field is rapidly expanding and new products are becoming available monthly.

Biotechnology and the Future of Cattle Practice

Interferon

Interferons have received a great deal of publicity because of their anti-proliferative (anti-cancer) activity. Athough interferons have proved useful in many types of cancer, they have not proved to be the "panacea" for cancer that the news media initially portrayed them to be. Another activity of interferon compounds that has long been recognized is their potent antiviral activity. Interferons have some advantages over vaccines in that they are active against a wide range of viruses, whereas vaccines are virus-specific. However, interferons have a very short half-life in the body. Therefore, for prophylactic use, interferons must be administered shortly before exposure to a virus. For therapeutic uses, repeated interferon treatments are usually recommended. The antiviral activity of interferons has been much more widely investigated for human use than for veterinary use. Many human viruses are susceptible to interferon both in laboratory trials *in vitro* and in clinical trials *in vivo*. Interferons also have many potential uses for bovine infectious diseases. There has been a great deal of interest in the use of interferons for prevention and/or therapy of viral infections associated with the bovine respiratory disease complex (Shipping Fever).

Recombinantly-derived human alpha interferon has

recently received FDA approval. This interferon is approved only for use in humans for treatment of hairy cell leukemia. Its use in cattle would constitute extra-label drug use. Naturally-produced ("native") human leukocyte interferon has been approved for veterinary use in the state of Texas *only*. Three products are presently on the market in Texas, one for cattle, one for horses, and one for small animals. For cattle, this interferon is approved for prophylactic treatment of shipping fever associated with IBR. For horses, it is approved for prophylactic and therapeutic treatment of viral induced respiratory infections. All of the interferon on the market for veterinary use is of human origin and is recommended by the manufacturer for oral use. Bovine interferons have been produced both by natural and recombinant techniques. A major drug company has invested in production of recombinantly derived bovine alpha interferon. This product is not yet on the market. It is unknown whether products of human or bovine origin will be superior to one another for use in cattle. It is also an unknown whether recombinantly-derived or naturally-produced interferons will prove most beneficial for use in cattle. Extensive clinical trials will be necessary to discover the full potential of interferons for veterinary use.

Interleukin-2

Interleukin-2 is a lymphokine (substance produced by a lymphocyte) that has recently received much media attention for use in human cancer treatment. This immunomodulatory substance (previously known as T cell growth factor) stimulates both T lymphocytes and B lymphocytes to proliferate and stimulates activity of two "cancer-killing" types of lymphocytes, NK cells and LAK cells. A commercial company has developed recombinant DNA techniques for production of bovine interleukin-2. Interleukin-2 has a short half-life in the body. Future possibilities for use of this substance in cattle may include use as a vaccine adjuvant.

Summary

There is clearly a revolution going on in the methodology which is used to develop new diagnostics, prophylactics and therapeutics for human and veterinary medicine. It is fortunate for the bovine practitioner that many of the first veterinary products developed using new biotechnology techniques have been for use in cattle. The possibilities for future veterinary products derived by these methods will be limited only by the financial and time resources committed to such endeavors by public research institutions and private industry.

Abstracts

Saturated fatty acid content of cow's milk through diet formulation

IN a duplicated 4 × 4 Latin square experiment, eight Friesian cows were given four isoenergetic diets consisting of hay, soya bean meal and either barley or oats in the approximate proportions 34:12:54 on a dry matter basis. The oats diet provided 537 g fatty acids per day, whereas the barley diet provided only 211 g per day. Milk yields were slightly higher on the oats diet, but there were no significant differences in the yield of fat. However, the milk fat of the barley fed cows contained only approximately 615 g saturated fat/kg, compared with 750 g/kg in the barley fed cows. There was a corresponding increase in the content of the mono-unsaturated fats in the milk fat of the cows fed the oats diet.

MARTIN, P. A. & THOMAS, P. C. (1987) *Proceedings of the Nutrition Society* **46**, 114A

Farm Animal Abstract

Effects of high dietary sulphur on cattle

TWELVE crossbred Hereford calves were divided into two groups and fed concentrate diets containing either 0·2 per cent or 0·75 per cent sulphur, with free access to hay and water for 85 days. There were no significant differences between the groups in terms of the activities of the enzymes glutathione peroxidase, glucose-6-phosphate dehydrogenase and acetyl cholinesterase or plasma aspartate aminotransferase, nor in blood selenium concentrations, bodyweight gains or clinical health. The results show that moderate increases in dietary sulphur concentration should not impair selenium or copper metabolism or cause related disorders in cattle.

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