What's In Store in Immunology: Immunomodulators, Genetic Engineering, New Vaccines

Charles C. Muscoplat, *Ph.D.*
Molecular Genetics, Inc.
10320 Bren Road East
Minnetonka, Minnesota 55343

Summarv

Two major developments in biotechnology, namely recombinant DNA and hybridoma technology, have been employed to address problems in animal health and production. In this article we review three means by which these technologies have been employed to improve animal production: 1) prevent losses from infectious disease by facilitating the development of efficacious vaccines and antitoxins; 2) increase growth and milk production by providing unlimited quantities of genetically-engineered animal growth promotants; and 3) increase the nutritional quality of animal feed. This paper will first review the basic features of these new biotechnological developments and then discuss the kinds of reagents that have been developed by these technologies and how they will benefit animal health and production in the future.

"Life is the evolution of molecular machines... The beauty of something is not the atoms that go into it, but the way they are arranged." Carl Sagan.

Introduction

Since prehistoric times, man has wondered at the influence of heredity. In his own offspring as well as those of domestic animals and fruits of the field, family resemblances have appeared and disappeared with a mysterious predictability. As civilization progressed, the rudiments of genetic manipulation were learned and passed on from teacher to student. Tablets from ancient Babylonia show a sophisticated awareness of horse pedigrees, and carvings from a later date indicate the cross-pollination of date palms. Genetic engineering had begun. Europe in the 19th century proved fertile ground for advances in understanding the genetic process. Jean-Baptiste Lamarck, a French scientist, suggested that an organism's characteristics were inherited from its parent cells. Half a century later, the Austrian monk Gregor Mendel discovered that heredity obeys precise statistical laws. Mendel theorized that plant characteristics result from paired carriers of heredity. These elements are today known as genes. The contributions of Lamarck and Mendel were explored and expanded upon during the next hundred years. DNA (deoxyribonucleic acid) ultimately was isolated as the carrier for heredity, and in 1953 scientists discovered its molecular structure. These revelations opened the door for an unprecedented explosion of scientific knowledge, the most recent of which includes the biotechnological developments of recombinant DNA technology or gene splicing (altering heredity by transplanting genes from one organism into another). Out of these scientific advances emerged today's burgeoning industry of genetic engineering—the directed manipulation of genetic materials to develop commercial products and processes. Further advances in immunology have been responsible for the development of hybridoma technology in which highly specific antibody molecules termed monoclonal antibodies have been responsible for an additional component of the new biotechnology.

The impact of these technologies has been far-reaching with the most rapid developments occurring in human pharmaceuticals and agriculture. In fact, developments in the agricultural field are occurring so rapidly that genetically-engineered products should be on the market before the end of the year. These developments will impact most heavily on improving animal production by a variety of approaches including: 1) reducing animal losses by preventing infectious disease by genetically-engineered efficacious vaccines and antitoxins; 2) increasing production of beef protein and milk through the use of growth promotants; and 3) improving the nutritional value of animal feed. In these treatise we will describe the basic features of these new biotechnological developments and discuss the ways in which they have already contributed, or will contribute in the future to the improvement of animal health and production.

Recombinant DNA and Hybridoma Technologies

Recombinant DNA technology, which is considered a modern day form of genetic engineering, is not a single discipline in itself but rather represents a fusion of ideas and

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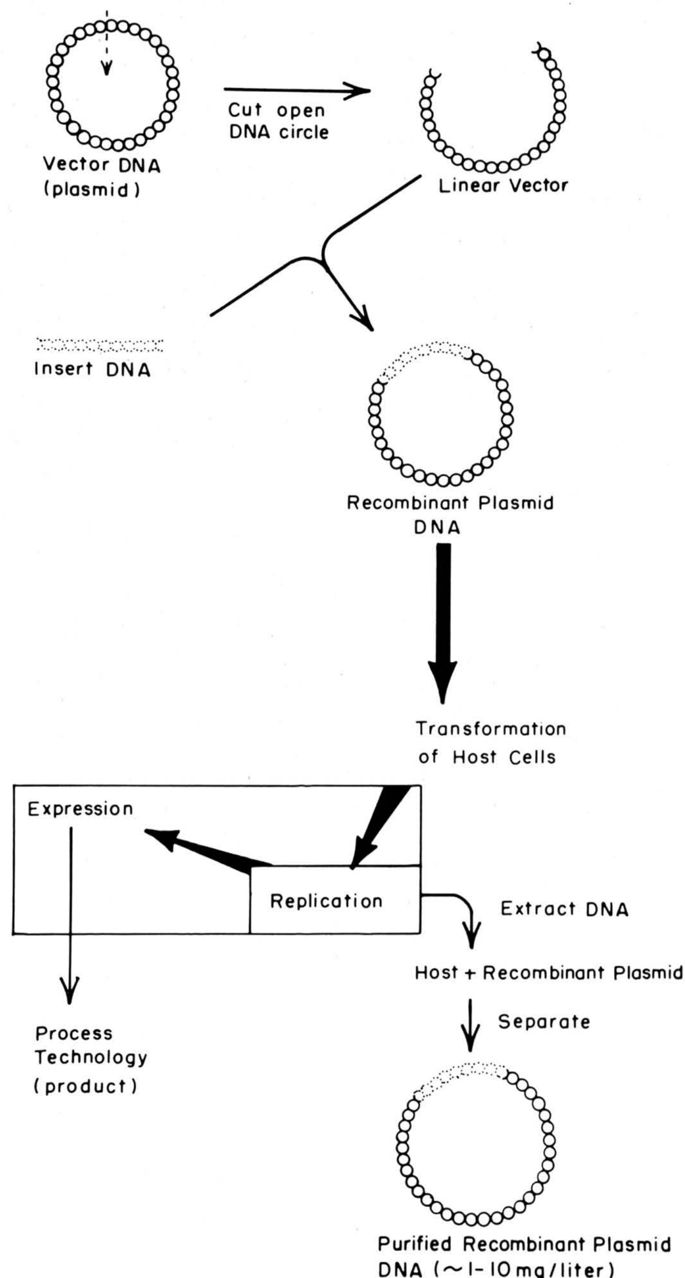
techniques from biochemistry, molecular biology, genetics and organic chemistry. It involves the restructuring and editing of genetic information and the construction of microorganisms with new genetic information. The technology is extremely powerful and allows one to isolate genes from any source (virus, bacteria, fungi, plants, animals), and amplify isolated genes to unlimited quantities at economical benefits through fermentation, and finally, manipulate genes by mutating or rearranging their components for the development of hybrid or novel gene products.

If a single breakthrough in gene splicing were to be identified it would have been the identification and isolation of specialized enzymes, termed restriction endonucleases, which act as biological scissors cutting chromosomes and DNA into unique pieces and enabling the isolation of specific genes or gene fragments. Since these restriction endonucleases make staggered breaks in DNA at sites exhibiting two-fold rotational symmetry the result is a piece of DNA with complementary cohesive ends which can then be, by virtue of these "sticky" ends, inserted or recombined with another piece of DNA that has been cut by the same enzyme. Since there are well over a hundred different restriction endonucleases and since each enzyme recognizes specific, but for the most part, different sites on DNA molecules, these enzymes can be used to cut DNA into a variety of pieces containing one or more of the gene(s) of interest.

The basic recombinant DNA experiment is depicted in Figure 1. The essential ingredients in this technology includes: 1) a DNA vector which generally represents the chromosome of either a plasmid, which are autonomously replicating DNA molecules found in bacteria and yeast, or a virus which can infect bacteria or higher organisms. Vectors must be able to replicate in living cells after foreign DNA is inserted into them; 2) a DNA fragment to be inserted into the vector; 3) a method of joining the insert DNA to the vector; 4) a method of introducing the joined molecules (recombinants) into a host that can replicate them; and, 5) a method of detection of those cells that carry the desired recombinant DNA molecule. Once the vector carrying the inserted foreign DNA molecule is placed into an organism such as a bacteria or yeast it will replicate to make many copies of itself and the foreign gene insert thereby providing an unlimited supply of the gene of interest. In order for the foreign gene insert to be expressed into protein in the bacterial cell certain specific features that are important to the bacteria's biosynthetic machinery must be available to the gene (Figure 2). For example, appropriate recognition signals for both bacteria-mediated transcription (RNA production) and translation (protein production) must be present.

In addition to recombinant DNA techniques two additional developments, both of which are in organic chemistry, have greatly facilitated progress in genetic engineering. The first involves chemical methods to synthesize genes or gene fragments *de novo* in an effort to modify or alter genes. These methods enable the gene to be created chemically from knowledge of the sequence of the amino acids in the protein encoded for by the gene of interest. The second development involves chemical methods to synthesize peptides and small proteins *de novo* allowing the generation of peptides containing active sites or antigenic determinants from knowledge of the nucleotide sequence of the gene. These developments have been employed recently to determine the specific regions of a viral protein molecule important in generating antibodies that neutralize infectious virus in an effort to make synthetic peptide vaccines.

Figure 1. A recombinant DNA Experiment



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The other major biotechnological development that will impact on animal health and production is hybridoma technology. This technique which results in the generation of monoclonal antibodies by cell fusion procedures will be useful for the diagnosis of specific diseases as well as the therapeutic prevention and cure of diseases affecting the morbidity and mortality of farm animals. Moreover, because of their tremendous specificity these monoclonal antibodies will be useful for the purification of various genetically engineered products following fermentation in bacteria or yeast. The procedure (Figure 3) basically involves fusing spleen cells from mice immunized with an antigen to which a monoclonal antibody is desired, to mouse myeloma cells in culture and screening fused cells for production of the specific monoclonal antibody with the labeled (radioactive or dye) antigen. The myeloma cells serve to immortalize the spleen cells so that they may be maintained indefinitely in cell culture. Special procedures are employed such as the use of myeloma cells requiring certain growth factors provided by the spleen cells fused over unfused myeloma cells. The monoclonal antibodies can then be obtained by marvesting the liquid medium from the cell cultures or by inoculating the fused cells into the peritoneum of mice and collecting the fluid present after ascites tumors have developed.

Figure 2. Typical Bacterial Gene Expression

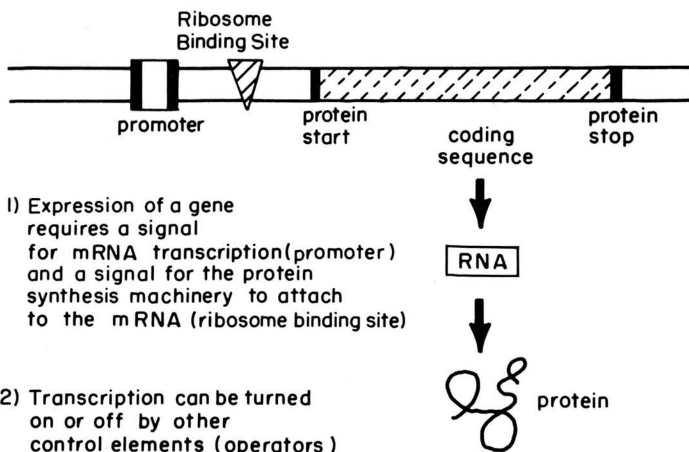
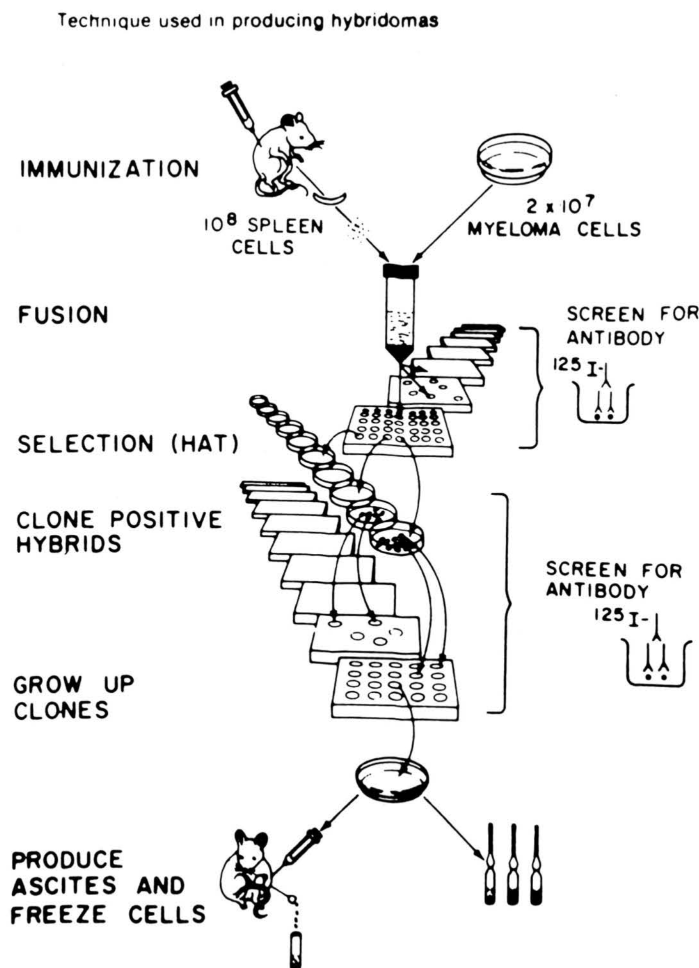


Figure 3.



Vaccines and Antitoxins

One of the major and earliest means by which recombinant DNA and hybridoma technologies will improve animal production will be to reduce morbidity and mortality from infectious disease by providing the animal health care industry with efficacious vaccines and antitoxins. Of the 45 million cattle born last year in the United States, approximately 10% died of infectious disease. Of the 94 million swine, up to 15% died of infectious disease. These losses occurred despite the use of conventional vaccines and large amounts of antibiotics. Since antibiotics are ineffective means of reducing the severity of diseases caused by viruses, many virus-induced diseases go unchecked because of either a lack of an appropriate vaccine or the availability of ineffective vaccine. Recombinant DNA procedures will enable the development of vaccines for infectious agents which grow poorly or not at all in cell culture thereby obviating the availability problem of these agents. Moreover, a genetically engineered subunit vaccine will not only exhibit the features of potency and efficacy, but also safety, ease of manufacture, and economy of production, as well. Immunologically, the genetically-engineered vaccine must be developed in such a fashion as to be administered in a single dose and induce immunity of long duration. It must protect against all serotypes in a given geographic region and must not induce adverse reactions. In addition to these biological features, a genetically engineered vaccine will exhibit several attractive manufacturing considerations including economy, long shelf life,

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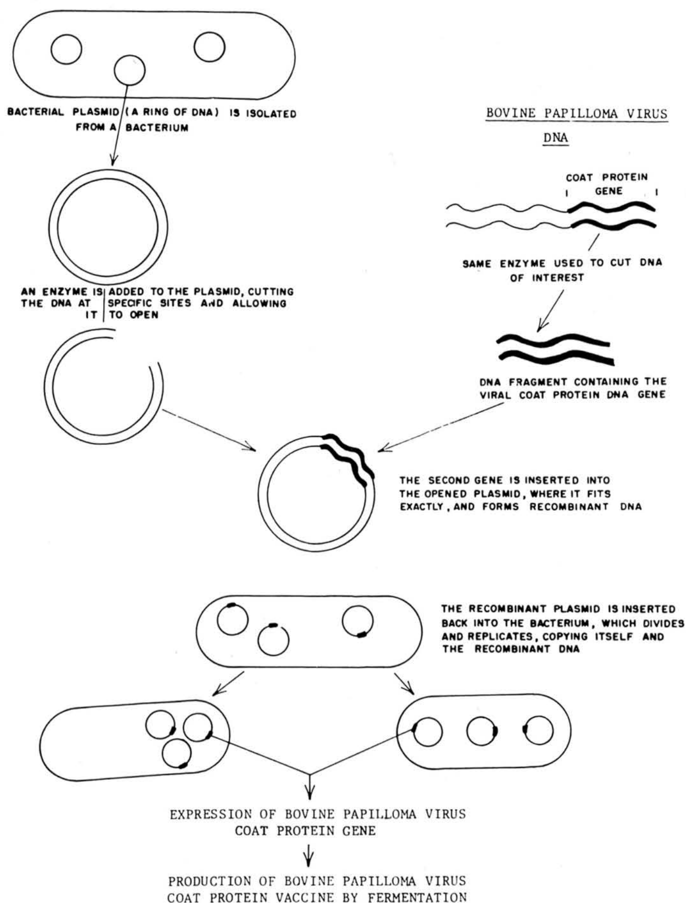
lack of infectious virus, and stability at ambient temperatures. The basic protocol for developing genetically engineered vaccine includes: 1) indentification of the major surface antigen of the pathogenic organism of interest which will induce antibody capable of neutralizing or inactivating the infectious organism; 2) identification of the surface antigen gene or its specific antigenic determinants; and, 3) isolation and transfer of this gene into a plasmid vector capable of expressing large amounts of its product in fermentable organisms such as bacteria or yeast (Figure 4). Such methodologies have been employed to generate large amounts of vaccine proteins against bovine papillomavirus, porcine parvovirus, canine parvovirus, foot-and-mouth disease virus and K99 *E. coli.* Although results on the potency and efficacy of these vaccines is presently awaiting completion of pre-clinical, clinical and field trials, preliminary tests on several of these genetically-engineered subunit vaccines indicates that they are excellent immunogens exhibiting all of the favorable features predicted.

Figure 4. Developing a papilloma virus vaccine by recombinant DNA techniques.



Monoclonal antibodies have also been generated for protection of newborn calves and swine against enteric collibacillosis responsible for neonatal diarrhea or scours. Although both conventional and genetically-engineered vaccines are available, the monoclonal antibody approach appears to be far superior to vaccination for two reasons. First, vaccination of the dam requires anticipating the problem, which may not be feasible. Second, vaccination of the dam requires maintaining breeding records since vaccination must be given twice, at six and two weeks prior to birth. Since scours usually occurs within the first 24 hours of life with susceptibility to the disease being markedly reduced after 24 hours of life, and the action of the pathogenic bacteria is restricted to the intestine, oral administration of a protective monoclonal antibody to provide passive immunity within 24 hours of birth serves to protect newborns from developing the disease on farms where the disease is prevalent. In both preclinical and clinical (Tables 1 and 2) testing to date a monoclonal antibody against the K99 strain of *E. coli* protected animals from lethal doses of challenge with the pathogenic strain of bacteria. The K99 - specific monoclonal antibody appears to be group specific capable of reacting with the adhesive entity on the pilus of over 100 strains of *E. coli.* Additional attributes of this monoclonal antibody includes reproducible specificity, high liter, and reduced costs. With the effectiveness demonstrated to date with this reagent and its ease of administration it should be an extremely useful product to curtail scours in calves. Similar monoclonal antibodies for two additional strains of pathogenic *E. coli* responsible for scours in newborn piglets (i.e. K88, K987) have also been developed and are presently in pre-clinical testing.

Growth Promotants

The development of natural growth hormones for livestock and poultry represents a major means for improving animal production, and, genetic engineering techniques have made this development a reality both for logistical and economic reasons. Several groups have now cloned bovine growth hormone (bGH) to expression in bacteria and yeast. The recombinant DNA procedures employed were similar to those utilized for cloning virus genes pertinent for subunit vaccination production. A mRNA species from pituitary gland enriched for bovine growth hormone nucleotide sequences is first reverse transcribed into DNA, and then this DNA copy is inserted into plasmids for expression in bacteria and yeast. Because this cow bovine growth hormone gene lacks the required regulatory features necessary for these microorganisms to express this gene, some additional restructuring of the gene is required. One of these maneuvers results in the addition of an additional amino acid (methionine) at the beginning of the bovine growth hormone gene so it differs slightly from naturally-occurring bovine growth hormones. Nevertheless,

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Table 1. Protection of newborn calves by the oral administration of a K-99 - specific monoclonal antibody.

Trial Group*	Calves	
	Alive	Dead
Monoclonal Antibody	9	1
Placebo	2	12

*Completed Trials to Date

Table 2. Protection of Newborn Pigs by the Oral administration of a K99 specific monoclonal antibody.

Trial Group	Pigs	
	Alive	Dead
Monoclonal antibody	11	3
Placebo	2	8

despite the presence of this additional amino acid at the beginning of genetically-engineered bGH, preliminary clinical studies have indicated that it is as effective as natural-occurring bGH in stimulation of milk production. For instance, whereas milk yields were increased 10.3% for natural bGH over a 6 day period of treatment, milk yields were increased by 12.9% for recombinant bGH. Neither milk fat, lactose nor protein percentages were affected by the treatment. Moreover, feed intake was also unaffected by treatment. Feed efficiency (Kg milk / Kg feed) was improved by 9.5% and 15.2% for natural and recombinant bGH, respectively, and no adverse effects were observed based upon body temperature and somatic cell counts. Thus recombinantly-derived bGH enhanced milk production and improved feed efficiency in a manner similar to the biological responses observed with natural bGH. Genetically engineered approaches to improving animal production appear to be directly applicable to hormones and other growth promotants where availability of the natural substance is limited and the costs to obtain the natural hormone exceed reasonable marketing considerations. Further studies are required to determine the safety of recombinant bGH for both treated animals as well as the consumer of its milk. An additional growth promotant presently under development is porcine growth hormone.

Feed Improvement

Certain agricultural crops, particularly corn, is used as a major source of feed for animals. Corn is an excellent source of energy but a poor source of protein for livestock feed. Poor protein quality is directly related to a deficiency of an essential amino acid such as lysine. In considering issues of protein quality it is important to distinguish corn feed to hogs and poultry and that feed to dairy and beef animals. Hogs and poultry have specific amino acid requirements whereas cattle as well as other ruminants do not. Hog and poultry farmers must therefore purchase additional protein to supplement a corn based ration. An improvement in protein quality that is, the relative amounts of essential amino acids, can have a pronounced effect on such factors as rate of gain and feed efficiency in hogs and poultry and in turn reduce the amount of supplement required to balance the ration.

Producing corn hybrids with high quality protein is a unique example of a product which has been developed using traditional plant breeding efforts and which was successful in terms of protein quality and improved nutritional value but failed because of unacceptable agronomic traits. In the early 60's a corn seed mutant, referred to as opaque-2, was discovered that exhibited an elevated lysine content. Since the altered amino acid composition was due to a single gene with an easily identified outward appearance (opaque-2 kernels do not transmit light whereas normal kernels do) plant breeders and geneticists immediately began to introduce this gene into the agronomically important inbred lines. In general, conversion of these lines and the resulting hybrids resulted in improved lysine content and nutritional value as demonstrated by feeding studies. The downfall of opaque-2 in the United States came as a consequence of depressed grain yields, poor seed quality, and greater susceptibility to insects and diseases attacking the grain. Thus conventional plant breeding procedures have resulted in corn hybrids with improved lysine levels but depressed yield and low levels of grain quality and pest resistance.

Recombinant DNA technology represents an important method for selection of specific desirable traits and exclusion of undesirable traits. In the case of corn it is known that 50% of the bulk protein of the corn kernel is a storage protein referred to as zein. The amino acid composition of the zein storage protein is low in lysine and tryptophan, two essential amino acids for man and monogastric animals, consequently these proteins influence the nutritional quality of the corn kernel. Recombinant DNA procedures have been utilized to isolate the zein gene and determine its precise biochemical structure. Knowledge of its gene structure resulted in the deduction of the primary structure of the zein storage protein. With the availability of this information it is now possible employing genetic-engineering approaches to alter the zein gene structure in an effort to increase its lysine content and therefore its nutritional quality. Once this has been accomplished the high lysine zein gene can be transferred back into corn cells and corn plants regenerated containing a high lysine storage protein.

Although the former task is still in the experimental stages the latter task is not since tissue culture procedures capable of plant regeneration initiated from juvenile tissues of corn

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have already been developed. Simply stated, tissue culture is the process whereby large populations of cells are stimulated by nutritional and hormonal conditions to grow continuously in a defined laboratory environment. Shoot meristems develop in large numbers in these cultures and under the proper conditions these meristems develop rapidly into complete plants which produce seed at maturity. In fact the tissue culture corn regeneration technology have been useful in isolating amino acid overproducer mutants of corn by virtue of the fact that these tissues will randomly undergo spontaneous mutations in cell culture. Recently overproducer mutants from the aspartate biosynthetic pathway (responsible for synthesis of lysine, threonine, methionine and isoleucine) in which threonine is increased approximately 100-fold have been isolated. This represents a 30-60% increase in the total threonine content of the kernel, an amount that would greatly improve the nutritional value of the grain if lysine and tryptophan were similarly increased. Analysis of the threonine overproducers indicates that they are inherited as dominant mutations and have the distinct advantage of creating very specific changes in the kernel (i.e. selectively increasing the concentration of specific amino acids) without causing unwanted pleiotropic effects in other kernel or plant characteristics. Thus from this one example of crop development it is clear that genetic engineering approaches will represent a viable means with which to improve the nutritional quality of corn for feed. Much of the efforts to successfully genetically engineer new strains of corn are directed at developing the appropriate vectors that will allow expression of genes such as zein to be produced at levels that will have a positive effect on the overall nutritional value of the seed. In a similar fashion, new strains of corn that offer resistance to disease, tolerance to herbicides, increased yields, and shorter maturation times will be developed.

Conclusions

Genetic engineering in agriculture will continue to be directed towards manipulation of microorganisms to produce animal vaccines, hormones, amino acids, and other chemicals or drugs with the ultimate aim of improving the quality, health and production of farm animals. Genetically engineered products such as vaccines, antitoxins, growth promotants and interferons (Tables 3 and 4) will be introduced into the veterinary marketplace in the near future. Many of these products are now being tested in animals.

Vaccines will be produced rapidly because there is a great need for new and improved vaccines for animals and because the regulatory requirements for animal vaccines are not as lengthy as those for human pharmaceutical products. There are a number of vaccines produced by conventional technology that are either expensive or unsafe. Other vaccines cannot be made at all using conventional technology. Vaccines for calf scours, Foot and Mouth Disease, feline leukemia, rabies, Rift Valley Fever, and numerous others are already under development.

The development of natural growth hormones for livestock and poultry will be a major area for genetically engineered products. Many believe that genetically engineered growth hormones hold greater potential for agriculture than even vaccines. Certainly these new growth hormones that mimic natural growth hormones will replace existing steroids or other growth promotants currently being used.

The feed industry will gain enormously from the developing genetic technology. Recombinant DNA will allow microorganisms to produce less expensive and more nutritious feed ingredients. Genetic engineering will eventually help increase crop yields, make possible more nutritious corn and other crops, and produce less expensive vitamins, amino acids and single cell protein.

Finally, there is the realm of antibiotics, where through recombinant technology, organisms that now produce in such low concentrations that it is not practical to recover the antibiotic, can be altered to produce much larger quantities for the marketplace. Similarly, some antibiotics are produced naturally in environments so hostile that the antibiotic is rapidly destroyed. It is quite possible to utilize recombinant DNA technology to produce these antibiotics from transformed organisms in environments that would not have the destructive quality of the natural one. The amplification of productive capability through recombinant technology could also be utilized to increase considerably the concentration of existing antibiotics in culture media, thus decreasing their cost and widening their availability.

In conclusion, it is evident that the industrialization of

Table 3. Products already developed by genetic engineering to improve animal health and production.

Vaccines —	Bovine papillomavirus, porcine parvovirus, canine parvovirus, Foot-and-Mouth disease Virus, K-99 (Scours)
Antitoxins	K-99, K-88, K-987
Growth Promotants	Bovine growth hormone, porcine growth hormone
Bovine Interferons	

Table 4. Future developments employing genetic engineering to improve animal health and production.

Better & more efficacious vaccines and antitoxins against many more diseases
Interferons
Additional animal hormones
Cheaper feed supplements, antibiotics
Fertility control, sexing
Improvements in animal feed

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recombinant DNA technology can lead to useful products and processes. Because this is a basic methodology, the unforeseen applications may very well be more important than any of those that have been proposed so far to date. The underlying science of molecular biology and molecular genetics is dynamic and it is reasonable to assume that new opportunities will be created as the depth of our scientific understanding increases. This new technology is surely no panacea. On the other hand, it carries the realizable potential of contributing significantly to the solution of some of the most difficult problems facing animal health and production today.

Panel Discussion

Question: The question is directed to Dr. Ardans regarding the use of modified live BVD viruses in dairy herds.

Answer: When I first got out of school that was a question that I thought I pretty well understood and I find myself, I think, as I go along saying more and more, I don't know. I've gone the full circle, I think, with BVD. Unless an outfit has had a significant problem with BVD, it hasn't been in our areas—I've been really pushing BVD vaccines. I know that I am going to get torn apart by some of the biologics people for this thing. But that has been our feeling. The inactivated ones, especially the ones using the Singer strain, I don't think have been out long enough really—I haven't made any decisions in my own mind about it. There's a lot of talk about which strain is the better strain to use and I guess I have not really seen anything that is definitive that says the NADL strain is better than the Singer, or the Singer is better than that. I think some of the problems that we had with some of the early vaccines were that there were some contaminating factors in some of those vaccines. I think that is where we got into some problems and I think that's the beauty of what Dr. Muscoplat was talking about. In the future we can produce, or hopefully produce, vaccines that will be noninfectious and hopefully not have the effect on the immune systems that BVD certainly in some instances seems to really have. I realize that's a long way around the bush saying I don't know, but I really don't have any firm recommendations on that. To take the last question, the question with the half-life of maternal antibodies being such as they are, the recommendations at times have been to prolong vaccination schedules until these antibodies have diminished and in many cases they're not. In certain diseases, persistence of paternal immunoglobulins have been shown for 6, 7, 8 or 9 months; 9 months would be stretching it somewhat, but BVD has been shown to go out that far. What about recommendations for vaccine? And the second one was, what antibody can be absorbed in the adult cow? My feeling is what antibody isn't absorbed in the adult animal because they don't have this mechanism that the young calf does so that they can absorb it orally. So I don't think it is an efficacious way. Going back to the immunization schedules, I think the situation that we get into with young calves, an area where we really need some good work to see what kind of schedules we might be able to develop for these baby calves, even though we give calves a vaccine, for example, and they don't necessarily make a response, in the serum that we can measure, there's probably pretty good evidence that those calves have been primed. So that when they are immunized the next time or they're given the vaccine there have already been some memory cells set up so that you get a quicker response. We have some trials that we are trying to get going now with some baby calves to see what type of intervals. I think we're probably going to come to using a multiple type approach or some thing, vaccinating over a more frequent schedule to see if we can get over the effect of the maternal immunity, and there's no doubt there is going to be some effect of the maternal immunity as far as inhibiting these organisms, say for a live virus, to go ahead and replicate. But there probably is going to be a little priming and that is the thing we are going to study. Lavamisole has been used. That is probably the one, if you want to call them immunomodulators, immunopotentiators, that are available that had been used in cattle. The problem is that we don't have from an experimental standpoint real good assays yet to really define what effects levamisole does have on say some of these subsets of the t-lymphocytes. Now in certain situations there have been good examples where levamisole seems to be associated with an increased t-cell response. But the thing to stress is that it seems to be only effective in those animals that have a t-suppression. It doesn't seem to do anything for the animal that seemingly has normal response. When you start looking at the area of how it affects antibody production, there are some individuals that have said they have seen better antibody production. There was an individual that was a graduate student at Davis a few years ago who used it along with an IBR vaccine trial and he actually saw a suppression in the antibody response in the particular situation when he used levamisole. So I think it can be used, but I don't think we fully understand it.

Second Answer: Let me just give two comments, maybe three. Dr. Don Johnson at the University of Minnesota, about 5 years ago, made some experiments on cattle that came into the veterinary clinic that really had what you would call chronic BVD, really non-responsive, and was able to get them to turn around with levamisole therapy. The information was never published but I believe it was sound. There was a recent report in the Amer. J. of Vet. Res., about 2-3 months ago, from Saskatoon showing that administration of levamisole to feedlot cattle significantly improved antibody responses to IBR. The third point would be that there are over 600 published reports in the literature documenting the immune-cementing properties of levamisole in the laboratory or some controlled situation. The problem would be to translate that into economic benefits to the cattle producer. It has been a most difficult thing to extrapolate. Given the fact that there

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