Biotechnology and Its Future in Bovine Medicine

Bennie I. Osburn *DVM, PhD*
School of Veterinary Medicine,
University of California
Davis, California

© Copyright American Association of Bovine Practitioners; open access distribution.

Introduction

The most significant technological advances that resulted in improvement in disease control in the last century include vaccinations and antibiotics. Vaccines became a popular method of disease control early in the century. In the late 1940's and early 1950's the introduction of antibiotics and pesticides had a dramatic effect on improving the health status of animals.[1] Although a significant improvement in animal health care occurred, disease was not eliminated. Diseases and the related animal production continue to cause an estimated $15 to $16 billion dollars a year loss in the United States alone. New methods and approaches are needed to significantly improve upon these disease losses. Recent advances in the areas of molecular biology, immunochemistry and genetics have brought a number of new approaches and hopes for significantly reducing animal health and disease problems. In this manuscript we will address the applications of these newer technologies to disease diagnosis, vaccinations and the immunomodulators that may improve animal health.

Disease Diagnosis

The traditional methods of diagnosing disease include clinical signs, pathological lesions, isolation of the causative agent, and/or serological test procedures.[1] Many times clinical signs may be nonspecific as a number of different infectious agents may cause similar signs. Similarly a set of lesions may be associated with the pathology of a number of diseases. Clinical and pathological parameters require the full expression of the disease. Subclinical forms of the disease or infection are usually overlooked in these situations. Isolation of organisms can be within 24 hours or after 4 to 6 months of culturing. In many instances, the methods currently in place are labor intensive. The most commonly used technique for confirmatory diagnosis is serology. This procedure indicates that an animal has been exposed to an agent, however it is not an indication that the agent is the cause of the disease at that time.

The recent advances in molecular biology and biotechnology are impacting the area of diagnostics in both human and veterinary medicine. Specifically, the use of radiolabeled or enzymatically labeled markers makes it possible to identify with great sensitivity molecular markers in tissues, in solutions or on solid matrices. These markers can be attached to nucleic acids or to antibodies that are used to identify genetic material or subunit antigens on microorganisms or for specific chemicals. Cloning of genes provides a means of rapidly propagating large quantities of specific nucleic acids which can be used as standardized reagents for diagnostic procedures. Similarly, monoclonal antibodies are highly specific reagents which are directed to specific epitopes on microbial agents or to specific chemical structures associated with toxic principles.

Keynote paper presented at the XVI World Buiatrics Congress, Salvador-Bahia-Brazil, August 13-17, 1990.

Detection Systems

Major advances in detection systems have had a significant impact on rapid diagnostic technology.[2] The principal detection systems in use today are the radioactive/nonradioactive and direct/indirect detection systems. These detection systems consist of signal generating molecules which are linked to ligand-specific biomolecular probes such as nucleic acids or antibodies. The enzyme/ radiolabeled molecules offer the means for evaluating the result of an assay. Most detection systems are multipurpose, that is they can be used on more than one probe. The specificity of the response lies with the nucleic acid or antibody probe.

Molecular Biochemistry and Diagnosis

Modern molecular biochemistry is proving helpful in providing more rapid and sensitive diagnostic techniques to detect infections in domestic animals. These techniques do not rely on the isolation and identification of a live replicating microorganism, but on the detection of highly specific molecular subunits of the inciting agent in tissues. These subunits may be either gene sequences specific for the microbe or specific protein epitopes on the protein components of the microorganism.

Biochemical structures

Polyacrylimide gels (PAGE) have made it possible to separate genomic and subunit proteins by molecular weight and charge. The migration distance in a gel is related to molecular weight and possible secondary structure of the genomic segments.[3] Genomic segments may be obtained from microorganisms by enzymatic digestion by restriction endonucleases or in the case of reoviruses by genomic dissociation at the time the viruses are released

from the nucleocapsid. This technique represents a means of fingerprinting; however it has proven very useful for determining biotypic characteristic of certain viruses and bacteria. For instance, the technique has been used to demonstrate reassorting gene segments in bluetongue viruses and to differentiate reproductive from respiratory strains of bovine herpes viruses.[4,5] This procedure is also used to identify plasmids that may transmit virulence or antibiotic resistance factors in bacteria.[6] PAGE is also used to separate microbial proteins. Again, the subunit proteins are separated based upon molecular weight, conformation and charge. This procedure permits one to determine the number of subunits associated with the microbe and their functional activities.[1]

Molecular Cloning of Microbial Genomic Material

Cloning of genetic material provides a means of propagating large quantities of a single gene that can be used for diagnostic purposes. Individual gene sequences can be isolated on PAGE cut out of the gel, eluted, copied and then spliced into a bacterial plasmid.[1] The plasmid is then exposed to bacteria. The bacteria with the plasmid incorporated into it undergoes replication. As the replication occurs, there is also replication of the foreign genes. Since bacteria replicate rapidly, it is possible to obtain large quantities of the isolated gene as well. The copied foreign gene can then be extracted from the plasmid and used for diagnostic purposes.

Genetic Probes as Diagnostic Tools

Cloned cDNA copies of individual genome segments are exact replicas of the original genomic segment from which they had been cloned. Genetic strands with complementary nucleotide sequences will hybridize to gene strands with similar nucleotide sequences. Hybridization can occur between complementary DNA and/or RNA genomic materials.[7,8] Hybridization conditions for DNA/DNA and DNA/RNA genetic hybridizations on solid supports have been standardized.

Molecular Genetic Diagnostic Tests

The cDNA probes can be used in four diagnostic tests, Northern and Southern Blots, dot blot hybridization and *in situ* hybridization. Northern blots can be used to identify RNA in cDNA/RNA hybridization assays. The most common use is for RNA viruses. Southern blots can be used for DNA in viruses or bacteria. Northern and Southern blots are performed following the placing of the test sample on PAGE and then electrophoretically transferring the genetic material to a solid matrix. The cDNA labeled probe is administered to the martix and if the complementary nucleic acids are present hybridization occurs and a signal appears on the matrix. Dot blots are performed by extracting the nucleic acids from tissues and applying directly to the solid matrix. The labeled probe is then administered and if the complementary nucleic acid sequences are present hybridization will occur. *In situ* hybridization utilizes the labeled probe by applying it directly to tissues. The probe will seek out complimentary nucleic acid sequences and hybridize to it within cells or tissues on glass slides.

A relatively new procedure known as the polymerase chain reaction (PCR) offers great promise for expediting the time required for a diagnosis.[9] The principle of the procedure is to use an oligonucleotide primer from a constant region of a gene. Extracted nucleic acid from tissue is mixed with the oligonucleotide primer. The primer will anneal to complementary nucleic acid of the microbe and then the bound nucleic acids are placed in a thermocycler along with a polymerase enzyme. The thermocycler will alternatively heat to 90°c and then cool to 30°c. This difference in temperature is important for separating the annealed necleic acids and to create new copies of the gene through the action of the polymerase. The result is a rapid amplification of the specific nucleic acid which can then be used for diagnostic purposes.[2] A single virus can be identified in a sample of blood by using this amplification system.[10]

Molecular Diagnosis of Specific Microbial Antignes

Another approach to modern molecular diagnosis involves the immunological idenification of specific epitopes on proteins.

Protein Dot Blot Assay

The assay involves dotting 1-2 ul of monoclonal antibodies onto nitrocellulose strips. After air drying and blocking with gelatin, supernatants from microbial cultures are incubated with the dots on the strips. The monoclonal antibody originally bound to the NC paper forms complexes with the specific protein in the test culture supernatant. A rabbit polyclonal antiserum to the test enriched supernatant is added followed by a biotinconjugated affinity-purified goat IgG antirabbit IgG. The presence of bound goat antirabbit is detected by an avidinperoxidase conjugate with appropriate substrate. A positive color reaction is an indication of monoclonal specific antigens in the test culture supernatant.

In situ Hybridization Assay:

Indirect immunoperoxidase procedures using both polyclonal and monoclonal antibodies have been successfully applied to detect microbial proteins in formalin fixed tissues. After fixing in formalin or by rapid freezing, tissue sections are washed, blocking steps applied, and the tissues are incubated with antimicrobial specific antibodies.

Immunoblotting Diagnostic Tests

Identification of microbial subunit proteins using a western blotting procedure has been successful. Extraction of microbial proteins are then applied to a PAGE which

© Copyright American Association of Bovine Practitioners; open access distribution.

results in separation of the subunit proteins. The proteins are then electrophoretically transferred (western blotted) to NC paper. The strips of NC paper containing the separated microbial proteins are blocked with gelatin and incubated with polyclonal antiserum to the microbial agent. Biotinylated affinity-purified anti-immunoglobulin and avidin/peroxidase conjugate are added to the reaction. The microbial specific proteins on the NC strip are visualized for a color reaction of the substrate. Monoclonal antibodies against microbial-specific epitopes may detect the subunit proteins on western blots or by immunoprecipitation.

Vaccination

Vaccination is one of the more important means of controlling disease. The term vaccine is derived from the fact that Dr. Edward Jenner noticed that milkmaids did not develop smallpox mainly because many developed a mild pox-like skin disease from the cattle they were milking. These cattle suffered from the condition cowpox.[11] The term "vaccine" is derived from the Latin word for cow. Vaccines may consist of either killed or live organisms, usually the live organisms have been attenuated so that they do not cause disease. Killed organisms have to be given in relatively large quantities to assure that sufficient antigenic mass is present to stimulate a good immune response. On the other hand modified live viruses can be given in small quantities, however, these viruses do have the potential for replicating in the host, thereby causing a mild to moderate infection. The amplification associated with this viral replication provides sufficient antigenic mass to stimulate a good immune response.

Although vaccines have proven to be quite successful there are a number of disadvantages associated with their use.[11] These disadvantages include the fact that if there are live organisms present within the vaccine, a mild or sometimes a severe disease can result following the administration of the vaccine. Secondly, animals vaccinated with whole organisms develop a full range of immune responses so it is difficult to utilize a serologic test to differentiate the vaccinated animals from those that recover from a clinical disease. Third, in certain instances some of the modified live virus vaccines will actually lead to persistent and latent infections. These latent viruses can periodically be shed and infect other animals, which in turn may develop clinical disease. Fourth, it is also possible that some of the viruses can be transmitted through semen, embryos, and eggs. Modified live viruses may also, during the course of their viremia, be picked up by insects and transmitted to other susceptible animals in an area. Fifth, in some instances the administration of these vaccines to pregnant animals will lead to congenital infections and in some instances death of the developing fetuses. Sixth, many of the vaccines, particularly some of the killed ones, do not have the complete array of antigens associated with the different serotypes or strains of a virus and as such they do not provide widespread protection. Seventh, modified live vaccines are not stable enough for use in areas where refrigeration is required to properly store these specimens.

The new approaches used in biotechnology have provided some innovative vaccines. It is now possible to develop subunit vaccines. These vaccines are made possible by the fact that recombinant DNA/RNA technology permits large scale production of the immunogenic surface proteins. It is also possible to synthesize short polypeptides that represent the immunogenic region of the antigenic portions of molecules. The vaccina virus recombinant system may incorporate into vaccina virus, genes coding for the immunogenic proteins of other pathogens. In some instances it is possible to delete genes in either viruses or bacteria that remove the virulent properties of these particular microorganisms.

Subunit Vaccines: An example of one virus on which many of the new technologies have been utilized in vaccine development is that associated with vesicular stomatitis virus. This virus is a member of the Rhabdovirus family and it causes infection in cattle, pigs, and horses. The usual clinical signs are associated with vesicualr lesions on the tongue and oral mucosa. In the United States there are two types of virus, New Jersey and Indiana, which cause infection.

The VSV virus is a single negative stranded RNA that codes for five messenger RNAs and the corresponding five proteins, which have been identified as G, L, M, N, and NS. The surface of the virus consists of many spikes containing the G glycoprotein. The G glycoproteins spikes are responsible for attachment of the virus to cells, which is an important first step in the initiation of the infection. The M protein is the matrix or membrane protein. The other proteins, L, N, and NS are closely associated with the genomic RNA and as such form the internal nucleocapsid.

In order to make an effective subunit vaccine it is important to identify the major surface proteins of the virus. It is these proteins to which the appropriate neutralizing or inactivating antibody responses are usually directed. With VSV the key protein is the G glycoprotein to which antibodies completely blocked the ability of this virus to cause infection. It is possible to purify this subunit protein and immunize cattle with this glycoprotein in Freund's adjuvant. By immunizing cattle it is possible to prevent infection at the time of challenge with infective virus. This clearly demonstrates that the G glycoprotein is the key antigen in initiating an effective immune response which would prevent subsequent infections. It is possible to develop vaccines for a limited number of animals utilizing this technique, however, it is costly and labor intensive. Therefore other approaches utilizing biotechnology have been developed for this particular virus. The use of recombinant DNA technology has made it possible to mass produce the G glycoprotein in large quantities.

© Copyright American Association of Bovine Practitioners; open access distribution.

Synthetic Peptide Vaccines: In order to develop effective synthetic peptide vaccines, it is important to determine amino acid sequence of the antigenic proteins. There is then a need to link together these amino acids to form a peptide chain. Amino acid sequence of the protein is coded by the DNA and RNA of the organism. Chemical and enzymatic techniques have been devised for determining the nucleotide sequence of DNA or RNA. By knowing the structure of the nucleotide coding for a protein, the genetic code can then be deciphered to derive the amino acid sequence of the protein. The sequence of amino acids and their local molecular attractions determine the secondary structure of the protein, and the relationship of the structure determines the final folding characteristics of the tertiary structure of a protein.

Computer programs are now available which provide a graphic display of tertiary structure of a protein once its crystalline structure is known. Immunogenic sites are likely to be on the surface of the protein and to be accessible to antibodies. Two principal types of antigenic sites include: (1) a continuous antigenic determinant made up of contiguous amino acids forming along the length of the primary structure, and (2) a discontinuous determinant made up of groups of amino acids separated from each other along the primary structure but brought into proximity by tertiary folding of the molecule.[11]

With computer graphics, it is possible to get an approximate idea of the portion of a protein that is likely to be on the surface, particularly if the primary structure is known. Hydrophobic amino acids are usually buried deep within the protein molecule, whereas the hydrophilic amino acids of lysine, arginine, aspartic acid and glutamic acid, are found on the outside of the molecule. Proteins rich in hydrophilic amino acids are likely to be on the surface. The amino a cid proline is associated with a sharp bend in the molecule at sites that are immunogenic. In order to determine whether a sequence is immunogenic requires knowledge about the crystalline structure of the protein, as well as the amino acid composition and the hydrophilic spots on the surface. Once these sequences and the folding pattern of the polypeptides are known, it is possible to synthesize through computer programming the amino acid sequences which will make up the comparable immunogenic molecules. It has been possible to synthesize several peptides in six areas of the G glycoprotein of VSV virus. Certain of these peptides protect mice against VSV challenge. In addition, synthetic peptides representing the hemagglutinin that make up the surface spikes of the influenza virus stimulating the formation of neutralizing antibodies have been developed.

Ideally it will be possible to develop synthetic peptide vaccines that are directed to conserved antigens that are present on viruses or bacteria. These conserved antigens should stimulate an immune response that would be effective against all variants of the microorganism that is causing the infection. As a result, a universal vaccine that would protect against all strains of an infectious agent would be available.

Vaccina Vector Vaccines: Vaccina is a virus that is antigenically related to the human smallpox or variola virus, as well as to the bovine cowpox virus.[11] The large size of this virus is now being used as an effective carrier in recombinant vaccines. Some newer vaccines are now using the vaccinia virus as a vector.[16-22] Genes from other viruses, such as vesicular stomatitis virus, rabies, rotavirus and rinderpest can be inserted into the genome of the vaccina virus.[15-22] When the vaccina vector is inoculated into an animal, the foreign gene is expressed as the virus replicates in the host cells. If the foreign gene is used as an immunogenic protein, the host develops antibodies against that protein and the animal becomes immune to both the vaccina virus and the virus from which the foreign gene was taken.

In order to utilize the vaccina virus as a vector it is necessary to create a plasmid which carries a chimeric gene containing a translocated vaccinia virus promoter region which is linked to the coding segment of a foreign gene. The chimeric gene is then incorporated into the vaccina virus genome by homologous recombination in tissue culture cells that have been transfected with the plasmid and infected with the wild type vaccina virus. Although nonessential regions of the vaccina virus genome can be used as the site of gene insertion, the thymidine kinase (TK) gene locus provides some advantages, because the recombinants are then TK^-, which distiguishes them from the wild-type TK+ virus. The TK^- pherotype provides a simple method for selection and also serves to attenuate viral pathogenicity.

In order to make a vaccina vector for VSV, a plasmid was constructed which carried the gene coding for the G glycoprotein.[19] This gene was flanked by sequences of the vaccina virus TK gene. This chimeric gene was introduced into tissue culture cells infected with wild-type vaccina virus so that recombination would take place between the vaccina DNA sequences flanking the G gene in the plasmid and homologous DNA sequences in the intact, replicating vaccina genome, thus creating a population of infectious vaccina virus recombinants carrying the VSV G gene. The inserted G gene inactivates the TK gene so that the recombinants are TK^-. It is possible to select the recombinants which express the chimeric G gene by incubating with 5-bromodsoxyuridine. This compound is metabolized by thymidine kinase to a lethal derivative; that is the wildtype vaccina virus particles carrying the active TK gene are inactivated. Particles carrying the chimeric gene, which have an inactivated TK gene, are preferentially selected. The G glycoprotein expressed in cells infected with recombinant vaccina virus is properly glycosylated and cannot be distinguished chemically and immunogenically from the authentic glycoprotein of VSV.

© Copyright American Association of Bovine Practitioners; open access distribution.

The VSV-New Jersey G glycoprotein in the vaccina virus recombinant is capable of protecting cattle against challenge infections.[19] Furthermore, the cattle develop neutralizing antibody to VSV-NJ. These vaccines could produce a very effective way of protecting livestock from this important disease.

The vaccina virus is a double-stranded DNA virus with a genome of approximately 180 kilobases. The host range of the virus is wide, including man, cattle, horses, swine, sheep, goats, mice, and monkeys. It has been demonstrated that as many as 25,000 base pairs of DNA can be removed from non-essential regions of the virus without affecting viral infectivity. The average gene is about 2000 kilobases, as such this implies that a vaccina virus recombinant could be instructed to carry as many as 12 to 15 immunogenic genes taken from a variety of viruses, bacteria or parasites.

The advantage of a recombinant vaccinia virus are that it will express a single gene coding for a single immunogenic protein much like a subunit vaccine. Secondly, there is amplification of the protein through replication of the vaccina DNA and a number of genes, possibly as many as 12 to 15 may be expressed in one vaccina recombinant. The lyophilized vaccina virus is stable and resistant to drying and heat and as such these vaccines could have a long shelf life at ambient temperature.

Gene Deletion Technology

It is now possible to delete specific genes which will detract from the virulence of organisms. This has been done with the pseudorabies in swine and also with salmonella organisms which affect cattle, sheep and swine. The thymidine kinase gene has been deleted in pseudorabies virus which then renders this virus of low virulence.[23] It is now a commercial vaccine in the United States manufactured by 3 different biologic companies. Aro-A transposon deleted salmonella have been developed and shown to be highly effective for salmonellosis in cattle.[24]

Immunomodulators

Through the use of biotechnology it is now possible to generate large quantities of natural immunomodulators. The immunomodulators, interferons and interleukins have been found to have beneficial effects on immune responsiveness.

Interferons:

Interferons have been recognized since the mid-1950's when they were first demonstrated to inhibit or greatly supress the ability of certain viruses to replicate in cell cultures.[25] Interferons are a group of small molecular weight proteins. The three major types of interferons are IFN-alpha, produced by leukocytes, as a result of stimulation by viruses or double-stranded RNA. IFN-beta produced by fibroblasts and epithelial cells in response to viruses and double-stranded RNA. The IFN-gamma is a product of T lymphocytes which have been stimulated by specific antignes or nonspecific mitogens. These IFN's have extensive antiviral, antiproliferative and immunoregulatory activities.[26]

Interferons are capable of inhibiting the replication of a wide variety of microorganisms, including bacteria, fungi, protoza, and both tumor and normal cells. IFN also regulates both humoral and cell mediated immune responses. High doses of IFN- are capable of suppressing the antibody-forming cells in response to antigens, whereas low doses appears to stimulate an increased activity of these same cells. IFN- also regulates the response of B and T lymphocytes to thymus-dependent and- independent antigens. Other immunoregulatory activities of IFN include enhancing the cytotoxic activity of natural killer cells and the phagocytic function of macrophages.[27,28] IFN's inhibit viral replication by two mechanisms; by blocking the initiation of protein synthesis and by degradation of viral mRNA. IFN also enhances the activity of NK cells and macrophage phagocytosis, inhibits delayed type hypersensitivity reactions, and regulates the production of antibody. IFN is also an effective regulator of the immune response, particularly of B and T lymphocytes, NK cells, and macrophages.

Although interferons can be induced by a number of methods, including the administration of many inducers, the production is quite low and not feasible for commerical purposes. Some inducers of IFN are toxic to human and animals, and IFN is usually metabolized very rapidly withing the body.

Recombinant DNA technology has made it possible to identify the genes that produce the different types of IFN, clone these genes, and mass produce the various subclasses of these particular proteins. These products can now be administered to animals at the time of vaccination, thereby boosting the antibody response. Administration at the time cattle are faced with stress lessens the chances of stress related shipping fever.

Interleukins:

Interleukins (IL) are produced by mononuclear communications which modulate immune responses. There are 6 interleukins IL-1 through IL-6 which have been identified. IL-2 has been cloned from cattle. Interleukin-2 is a glycoprotein produced by T helper cells.[28,29] IL-2 plays a very important role in promoting the clonal expansion of T lymphocytes that have been activated with antigens or mitogens.[30] IL-2, along with IFN-gamma and IL-1, a product of macrophages are all essential for differentiation and proliferation of cytoxic lymphocytes. IL-2 and IL-1 are also important for antibody production by B lymphocytes. In some instances IL-2 has now been used for long-term cultures of lymphoctyes in a number of different species.

In vitrio it is possible to add IL-2 to cultures to generate cytotoxic T lymphocytes, maintain long-term prolifera-

© Copyright American Association of Bovine Practitioners; open access distribution.

tion and cytotoxic cell cultures, stimulate natural killer cell activity, and assist in overcoming some immune dysfunction in lymphocytes taken from individuals with selected immune deficiencies.[2] There is some evidence that IL-2 synthesis may be suppressed by the presence of viruses. Glucocorticoids that depress immune responses can inhibit IL-2 synthesis. On the other hand, it is possible to override the effects of glucocorticoids by adding exogenous IL-2.

Recombinant DNA technology has now permitted the cloning of IL-2 genes from a number of different species. Human, bovine, mouse, as well as IL-2 from other species have been cloned. It has been demonstrated that both bovine and human IL-2 are capable of augmenting immune responses in cattle.[31] The human IL-2 has been shown to be effective in other species, such as the horse, pig, dog, cat, etc. The responsiveness of these various species to human IL-2 varies to some extent. The addition of these products, along with vaccines sometimes assist in boosting immunological responses to various antigens.

Conclusion:

Rapid advancing tehnological procedures directed at subunit proteins and genetic material are increasing the specificity of diagnostic procedures. The use of novel marker systems which can be amplified through bridging molecules has increased the sensitivity of these systems. Many of these procedures are resulting in animal side tests. This is greatly increasing the use of tests making it possible for veterinarians to make diagnosis in the field. New improved vaccines produced through rDNA, gene deletion or synthetic peptide technologies are providing new approaches for safe, effective vaccines.

References

1. Squire, K.R.E., Stott, J.L., Dangler, C.A. and Osburn, B.I. Prog. Vet. Microbiol. Immun. 3:235-250, 1987. 2. Dangler, C.A. and Osburn, B.I. In Biocatalysis in Agric. Biotech. J.R. Whitaker and P. Sonnet eds. Am. Chem. Soc. 389:230-241, 1989 3. Walker, P.J., Mansbridge, J.N. and Gorman, B.M. J. Virol. 34:583-591, 1980 4. Squire, K.R.E., Osburn, B.I., Chuang, R.D. and Doi, R.H. J. Gen. Virol. 64:2103-2115, 1983. 5. Engels, M.E., Steck, F. and Wyler, R. Arch. Virol. 67:169-175, 1981. 6. Hirsh, D.C., Ikeda, J.S., Martin, L.D. et al. Avian Diseases Vol 27, No. 3, July-September, 1983. 7. Wahl, G.M., Stern, M., Stark, G.R. Proc. Nat. Acad. Sci. 76:3683-3687, 1979. Thomas, P.S. Proc. Nat. Acad. Sci. 77:5201-5205, 1980. 9. Saiki, R.K., Schoaf, S., Foloona. F., Mullis, K.B. et al. Science 230:1350-1354, 1985. 10. Ou, C.Y., Kwok, S., Mitchell, S.W., Mack, D.H. et al. Science 239:295,297, 1988. 11. Yilma, T. et al Vet. Clinics of North Am: Food An. Pract. 1:419, 1985. 12. Lerner, R.A. Nature 299:529, 1982. 13. Lerner, R.A. Sci. Amer. 248:66, 1985. 14. Wilson, I.A. et al. Cell 37:767, 1984. Brown, F. Advances in Vet. Sci. and Comp. Med. J.L. Bittle and F.A. Murphy eds. Acad. Press Inc. 33:173, 1989. 16. Mackett, M. et al. Proc. Natl. Acad. Sci., USA 79:7415, 1982. 17. Mackett, M. et al. J. Virol. 49:857, 1984. 18. Smith, G.L. and Moss, B. Biotechniques 2:306, 1984. 19. Mackett, M. et al. Science 227:433, 1985. 20. Paoletti, E. et al. Proc. Natl. Acad. Sci. USA 81:793, 1984. 21. Andrew, M.E. et al J. Virol. 61:1054, 1987. 22. Collett, M.S. Advances in Vet. Sci. and Comp. Med. J.L. Bittle and F.A. Murphy eds. Acad. Press Inc. 33:109, 1989. 23. Kit, S. Proc. 90th Ann. Mtg: U.S. An. Health Assn., 1986. 24. Dougan, G. et al. Advances in Vet. Sci. and Comp. Med. J.L. Bittle and F.A. Murphy eds. Acad. Press Inc. 33:271, 1989. 25. Issacs, A. and Lindermann, J. Proc. R. Soc. Lond. (Biol.) 147:258, 1957. 26. Grossberg, S.E. N. Eng. J. Med. 287:13,79,122, 1972. 27. Gills, S. and Smith, K.S. Nature, 268:159, 1977. 28. Altman, A. and Dixon, F.J. Adv. Vet. Sci. and Comp. Med. J.L. Bittle and F.A. Murphy eds. Acad. Press 33:301, 1989. 29. Smith, K.A. Immunol. Rev. 51:337, 1980. 30. Smith, K.A. and Ruscetti, R.W. Adv. Immunol. 31:137, 1981. 31. Stott, J.L. et al. Vet. Immuno. and Immunopath. 13:31, 1986.

© Copyright American Association of Bovine Practitioners; open access distribution.