How DNA Studies May Advance Knowledge of Pathogenesis and Immunity

Gary Splitter, Tom Leary and Yi Gao, Department of Animal Health and Biomedical Sciences
1655 Linden Dr., University of Wisconsin-Madison, Madison, WI 53706

No other health profession than veterinary medicine must understand the health needs and individual disease states of such a diverse number of animal species. How can the health care of domesticated animals be rapidly improved to provide a reliable food source without the age-old threat of epidemics or severe economic loss to producer groups? Several fruitful areas of investigation may produce the answers to improving the well-being of animals.

First, new alternative vaccines may provide immunization that is more specific to the major epitopes of the pathogen, or combinations of protein material from several pathogens may be combined to produce an efficient hybrid vaccine. Identifying these important epitopes may utilize cloned and expressed individual genes from pathogens and cloned immune cells. Such techniques may bring new vaccines to domestic animals.

Second, exploration of the immune systems in animals may indicate how the body can be immunized to provide long-lasting protection, and whether individual animal species differ in their immune repertoires for combating infections. A deep appreciation of how immune cells of some individuals protect against bacteria, viruses, and parasites while the immune systems of others fail to protect will provide the foundation for improving the immune system or vaccination regimes. Surely, this understanding will involve the characterization of cell surface molecules on immune cells and characterization of the cytokines they produce.

Third, as molecular biological techniques are applied to the discipline, there is increased opportunity to construct new vaccines or create domestic species that are genetically resistant to one or more infectious disease agents. Insertion of particular genetic sequences in embryos for the purpose of constructing transgenic animals may offer new horizons in stimulating or supplementing the immune system. Therefore, as new frontiers in protection from disease arise, the impact these changes may have on the well-being and relation among animals and their environment will need closer examination. The following example with bovine herpesvirus-1 (BHV-1), a serious pathogen of cattle, illustrates how the immune system recognizes certain aspects of viral proteins and how a single viral protein can immunize cattle against bovine herpesvirus-1 challenge.

BHV-1 is an α-herpesvirus. Other members of this subfamily include human simplex virus, pseudorabies virus, and equine herpesvirus. Like these viruses, BHV-1 afflicts both the respiratory and genital tracts. Because herpesviruses persist in the presence of specific antiviral antibody, cell-mediated immunity is thought to be the more important mechanism in viral elimination upon exposure. This idea is supported by the fact that T lymphocyte activity can be demonstrated against viral surface antigen gB, gC, and gD of HSV-1 in both human and murine models, and adoptive transfer of immune T cells in mice can provide protection from lethal HSV-1 challenge. HSV-1 gB and gD as well as similar BHV-1 gI and gIV proteins have roles in viral attachment and penetration, respectively.

Given the overwhelming evidence that T lymphocytes recognize the primary sequences of protein antigen, it was our hypothesis that lymphocytes previously primed to antigen would recognize and proliferate to the nonglycosylated peptides synthesized in vitro. Therefore, we cloned viral-surface proteins gI (gB), gIII (gC), and gIV (gD) of BHV-1 into the encephalomyocarditis virus (EMCV)-derived transcription vector pE5LVPO. RNA transcribed from this vector are chimeric messages between the cloned gene and the 5' noncoding region of EMCV. Here we describe the cell-free synthesis of these viral antigens and the responses of T lymphocytes to each produced protein.

After cloning BHV-1 gI, gIII and gIV into the vector pE5LVPO, viral mRNA was transcribed. The transcribed mRNA was translated in rabbit reticulocyte lysates and each cloned

viral protein specifically stimulated bovine T lymphocytes previously primed to BHV-1. In addition, a panel of specifically truncated peptides has allowed the identification of at least two T lymphocyte epitopes encoded within gIII and one epitope within gIV. Also, evidence is provided to suggest that this new technology will be invaluable to the rapid identification of T lymphocyte epitopes and should allow epitope mapping to be more practical for a large number of antigens. Identifying viral proteins with T lymphocyte epitopes will permit engineering vaccines free of potentially suppressive viral components, a concern with BHV-1.

In addition, the desire to obtain authentically glycosylated viral protein products in sufficient quantity for immunological study has led to the use of eukaryotic expression vectors for protein production. A further advantage is that these protein products can be studied individually in the absence of their native viral environment. We have cloned a cDNA encoding BHV-1 gI in the eukaryotic expression vector, pZipNeo SVX1. Since this protein is normally embedded within the membrane of BHV-1 infected cells, we removed sequences encoding the transmembrane domain of the native protein. After transfection of the plasmid construct into the canine osteosarcoma cell line, D17, or Madin-Darby bovine kidney (MDBK) cells, a truncated BHV-1 gI was secreted into the culture medium as demonstrated by radioimmunoprecipitation and sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Both a $CD4^+$ T lymphocyte cell line specific for BHV-1 and freshly isolated T lymphocytes could recognize and respond to the secreted recombinant gI.

Using recombinant BHV-1 gI as a vaccine, both antibody and cellular responses were elicited in cattle. Nine of 10 cattle immunized with the recombinant truncated gI were protected when challenged with virulent virus by nasal aerosolization, the natural route of infection. Thus, this recombinant vaccine approach offers considerable promise as a protective immunogen without concern for latency and viral recombination associated with a modified live virus vaccine. Comparison of antibody responses between these recombinant vaccinated animals and killed virus vaccinated animals indicated that recombinant vaccinated animals produced high titered antibodies to gI, while killed virus vaccinated animals produced little antibody to gI. Because gI and gIV proteins are acknowledged to be important for protection, this experimental recombinant vaccine may offer an advantage over present killed-virus vaccines.

Using cattle immunized with commercial killed-virus vaccine, we have demonstrated specific $CD4^+$ T lymphocyte proliferation to UV-inactivated BHV-1 and the recombinant gI protein. On the basis of lymphocyte proliferation, interferon-γ production and serum antibody virus-neutralization capacity, animals within this population could be divided into either high or low responders. This response appears BHV-1 specific because proliferation to unrelated stimuli is unaffected. Using polymerase chain reaction and bovine specific oligonucleotide primers, $CD4^+$ BHV-1 specific cells from the high responding animals synthesized mRNA for IL-2 and IL-4, while $CD4^+$ BHV-1 specific cells from the low responding animals did not. This study provides evidence for distinct $CD4^+$ T lymphocyte subpopulations between high and low responding phenotypes to BHV-1 in vitro, results which may be important to understanding immunity to this important viral pathogen. Whether similar high and low responder cattle occur with recombinant gI protein is presently under study.

SUMMARY

Cattle immunized to BHV-1 recognize only particular segments or epitopes of viral glycoproteins. We have identified T lymphocyte epitopes present on gIII and gIV. Using recombinant gI protein lacking the transmembrane segment, cattle vaccinated with this single protein were protected against virulent virus challenge. T lymphocytes from vaccinated cattle can be categorized into high and low BHV-1 responder animals and the cytokines produced by these animals indicate distinct $CD4^+$ T lymphocyte subpopulations according to the responder status of the animal.