Immunomodulation in Bovine Respiratory Disease

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Introduction

Animals are constantly exposed to the threat of bacterial, viral, and parasitic infection and neoplastic disease. The immune system is responsible for warding off these threats so that the animal remains healthy. The fact that the normal state of existence for animals is health attests to the remarkable efficiency of the immune system. When some factor(s) interferes with normal immune function or when an animal is exposed to an overwhelming number of an infectious agent, disease occurs.

The basic understanding of the immune system is advancing rapidly and a picture of a highly regulated, complex system of cellular and molecular interactions is emerging. These breakthroughs in basic understanding promise to give scientists the means for manipulating the immune system to prevent disease from occurring rather than treating disease after it occurs. Advances in basic immunology have led to the identification of several compounds which show promise as immunomodulators. Immunomodulators are compounds that can "modulate" or enhance the function of the immune system and are sometimes called biological response modifiers. There are two basic types of immunomodulators: exogenous and endogenous. The exogenous immunomodulators include bacteria or bacterial derived products (e.g. Bacillus Calmette-Guerin (BCG), endotoxin, *Propionobacterium acnes)* and synthetic chemicals (e.g. levamisole and lipoidal amines). One mechanism of action of the exogenous immunomodulators is to induce the release of endogenous immunomodulators. The endogenous immunomodulators include proteins that are produced and secreted by cells (cytokines). Some examples of these proteins include interferons (IFN), interleukins (IL), tumor necrosis factors (TNF) and colony stimulating factors (CSF) (20). Genetic engineering techniques offer the potential to produce these compounds inexpensively. This manuscript focuses on research conducted at Iowa State University aimed at evaluating the potential for using immunomodulators in prevention and therapy of bovine respiratory disease. Research on immunomodulators in cattle has also been conducted by a number of other research groups, but will not be reviewed here.

Immunomodulators in Bovine Respiratory Disease

Vaccines and antibiotics for the prevention and treatment of the bovine respiratory disease (BRD) complex have reduced economic losses, but BRD is still the most costly disease of feedlot cattle in North America. It has become apparent that a major component in the pathogenesis of BRD is immunosuppression due to stress and/or viral infection. The lungs of these immunocompromised animals are susceptible to infection by strains of bacteria which possess virulence factors that further impair host defense mechanisms. Effective immunomodulating compounds which could overcome the immunosuppression associated with BRD should significantly reduce the economic loss associated with BRD in North America. At Iowa State University, our approach to developing an immunomodulator for use in BRD was to first attempt to define the cellular and molecular aspects of the immunosuppression associated with BRD. We chose to concentrate our effort on the neutrophil, a highly active phagocytic cell with a primary role in resistance to bacterial infection in the lung. Other cells of the immune system are also very important in resistance to BRD and have also been shown to be suppressed in association with BRD.

We, and others, have found that cortisol (which is increased by stress), other glucocorticoids, and several respiratory viruses (IBR, BVD, and PI-3) each induce defects in neutrophil function (1,8,10,11,15,16). The cellular and molecular aspects of neutrophil dysfunction differs with each inducer. The bacterial agents which cause the severe economic losses associated with BRD (*Pasteurella haemolytica, P. Multocida,* and *Haemophilus somnus*) have also been shown to actively interfere with selected aspects of neutrophil function (2,6,18).

The next step, after characterizing the defects in neutrophil function associated with BRD, was to evaluate potential immunomodulators. Some immunomodulators have little or no activity in normal animals but are effective in immunosuppressed animals. We therefore decided to evaluate the immunomodulators in both normal and immunosuppressed cattle. We chose to use dexamethasone, a potent glucocorticoid, as an immunosuppressant in our modle. It gives a reproducible immunosuppression and alters nearly all of the neutrophil functions which are altered by stress, viral infections, or bacterial virulence factors. However, the molecular mechanisms of dexamethasone-induced inhibition of neutrophil function are likely to be different from the mechanisms used by viruses or bacteria.

Our standard protocol has been to administer the compound to be evaluated concurrently with dexamethasone and to evaluate neutrophil function before, during, and after treatment. We then compare the results to those of a control group and a group which received only dexamethasone. The first compounds tested using this model were ones which were already approved for use in cattle for other purposes and were speculated to have immunomodulatory activity as well (levamisole, thiabendazole, ascorbic acid, and vitamin E-selenium) (12,14,17). None of these compounds convincingly altered the effects of dexamethasone on neutrophil function at reasonable dosages. One compound which did prevent most of the effects of dexamethasone on neutrophil function in vivo was avridine (a lipoidal amine also referred to as CP20,961) (13). Avridine is known to induce interferon production in vivo and probably also induces other biologic response modifiers. The avridine, however, was unacceptable for clinical use in the formulation which was evaluated because it induced swelling at the injection site.

The avridine results suggested that interferon or some other endogenous biologic response modifier may be capable of modulating neutrophil function. The next step was to attempt to identify the molecule(s) induced by avridine which enhanced neutrophil function. Since avridine may stimulate lymphocytes in vivo to secrete lymphokines (including interferon), we decided to evaluate the in vitro effects of lymphokine preparations on neutrophil function. The pattern of influence of the lymphokine preparation (which contained gamma interferon) on neutrophil function in vitro was similar to that of avridine in vivo (7). When we then tested recombinant bovine gamma interferon (supplied by Ciba-Geigy, Basel, Switzerland) for its in vitro effects on bovine neutrophil function, we found that it could account for most of the biologic activity of either the lymphokine in vitro or the avridine in vivo (21).

These results implied that the biologic activity of avridine could have been due primarily to the induction of gamma interferon. If this was the case, then the administration of recombinant bovine interferon gamma should have similar in vivo biologic activity as avridine. Three dosages of recombinant bovine interferon gamma (Ciba-Geigy Limited, Basel, Switzerland) were evaluated in normal non-immunosuppressed animals (9). The optimal dosage was selected and tested in an experimant having 5 controls, 5 animals immunosuppressed with dexamethasone, 5 animals treated with 0.5 mg of recombinant bovine interferon gamma, and 5 animals given dexamethasone plus 0.5 mg of recombinant bovine interferon gamma. The dexamethasone and recombinant bovine interferon gamma were each administered 2 days in a row. Neutrophil and lymphocyte function were evaluated twice before drug administration (to ensure that there were no major differences between groups before treatment) and 2 days in a row beginning 24 hours after the first administration of drug. The experiment was replicated in an additional twenty head of cattle so that there were ten animals per treatment group. The interferon gamma was found to have nearly the same activity in dexamethasone-treated animals as did the avridine (9,13).

Since the recombinant bovine interferon gamma was successful in improving several of the dexamethasone-induced defects in neutrophil function, it was decided to test the recombinant bovine interferon gamma in a bacterial infection model which depended upon dexamethasone immunosuppression as an important component of the pathogenesis. The bacterial challenge model used involved the intratracheal administration of 5 x 10^9colony forming units of *Haemophilus somnus* and the intramuscular injection of dexamethasone daily for 3 days starting 1 day before *H. somnus* infection. (3). This challenge regimen was selected because the bacterial challenge inoculum would not produce severe pneumonia in normal non-immunosuppressed calves, but would produce severe pneumonia in dexamethasone-treated animals. Therefore, an immunomodulator which could overcome the influence of dexamethasone should significantly decrease the severity of pneumonia.

In addition to the immunosuppression induced by dexamethasone, this model system involves at least two other factors that contribute to the pathogenesis. Young calves (less than 5 months of age) are known to have suboptimal neutrophil function (5,19). Secondly, *H. somnus* is known to have surface components which inhibit phagosome-lysosome fusion in neutrophils (2,6) and are therefore able to resist killing by the neutrophil (4). Therefore, stress-induced increases in cortisol concentration, suboptimal function of neutrophils in young calves, and *H. somnus* virulence factors which suppress neutrophil function may all contribute to the pathogenesis of *H. somnus* pneumonia. If an immunomodulator can improve neutrophil function (and/or other host defense mechanisms) in the face of any or all of these factors, it should reduce the severity of pneumonia.

Twenty-four Holstein steers (1-2 months of age) were used for this experiment, 6 served as nontreated controls, 6 received recombinant bovine interferon gamma (2 ug/kg body weight) subcutaneously daily for 2 days starting 1 day before infection with *H. somnus*, 6 received dexamethasone (0.04 mg/kg of body weight by i.m. injection daily for 3 days starting 1 day before experimental infection), and 6 received both the dexamethasone and recombinant bovine interferon gamma dosage regimens (3). The animals were monitored for one week, then necropsied to determine the extent of bacterial pneumonia. Two of the 6 dexamethasone-treated animals died due to bacterial pneumonia before the scheduled necropsy. The dexamethasone-treated group had a significantly increased total volume of affected lung tissue when compared to the control animals. The group which received gamma interferon only had pneumo-

nia which was essentially equal to that in the control animals. This was a mild pneumonia which was resolving at the time of necropsy, 7 days post challenge. The group which received dexamethasone plus recombinant bovine interferon gamma had pneumonic lesions essentially equivalent to those of the control group; the dexamethasone plus recombinant bovine interferon gamma group did not have the severe pneumonia observed in the dexamethasone treated group. Therefore, the recombinant bovine interferon gamma overcame the increased susceptibility to bacterial pneumonia induced by the administration of dexamethasone. This clearly demonstrated that gamma interferon can have a role in the prevention of bacterial pneumonia associated with immunosuppression without the involvement of a viral component.

References

1. Briggs, R.E., Kehrli, M. and Frank, G.H. Effects of infection with parainfluenza-3 virus and infectious bovine rhinotracheitis virus on neutrophil functions in calves. Am. J. Vet. Res. 49,682, 1988. 2. Chiang, Y.-W., Kaeberle, M.L. and Roth, J.A. Identification of the suppressive components in *Haemophilus somnus* fractions which inhibit bovine polymorphonuclear leukocyte function. Infect. Immun. 52,792, 1986. 3. Chiang, Y.-W., Roth, J.A. and Andrews, J.J. Influence of recombinant bovine interferon gamma and dexamethasone of pneumonia in calves due to *Haemophilus somnus*. Am. J. Vet. Res. In Press, 1989. 4. Czuprynski, C.J. and Hamilton, H.L. Bovine neutrophils ingest but do not kill *Haemophilus somnus* in vitro. Infect. Immun. 50,431, 1985. 5. Hauser, M.A., Koob, M.D. and Roth, J.A. Variation of neutrophil function with age in calves. Am J. Vet. Res. 47,152, 1986. 6. Hubbard, R.D., Kaeberle, M.L., Roth, J.A. and Chiang, Y.W. *Haemophilus somnus*-induced interference with bovine neutrophil functions. Vet. Microbiol. 12,77,1986. 7. Lukacs, K. Roth, J.A. and Kaeberle, M.L. Activation of neutrophils by antigen-induced lymphokine with emphasis on antibody-independent cytotoxicity. J. Leukocyte Biol. 38,557, 1985. 8. Roth, J.A., Bolin, S.R. and Frank, D.E. Lymphocyte blastogenesis and neutrophil function in cattle persistently infected with bovine viral diarrhea virus. Am. J. Vet. Res. 47,1139, 1886. 9. Roth, J.A. and Frank, D.E. Recombinant bovine interferon gamma as an immunomodulator in dexamethasone-treated and nontreated cattle. J. Interferon Res. 9,143, 1989. 10. Roth, J.A. and Kaeberle, M.L. Effects of in vivo dexamethasone administration on in vitro bovine polymorphonuclear leukocyte function. Infect. Immun. 33,434, 1981. 11. Roth, J.A. and Kaeberle, M.L. Suppression of neutrophil and lymphocyte function induced by a vaccinal strain of bovine viral diarrhea virus with and without the concurrent administration of ACTH. Am. J. Vet. Res. 44,2366, 1983. 12. Roth, J.A. and Kaeberle, M.L. Effects of levamisole on lymphocyte blastogenesis and neutrophil function in dexamethasone-treated cattle. Am. J. Vet. Res. 45,1781, 1984. 13. Roth, J.A. and Kaeberle, M.L. Enhancement of lymphocyte blastogenesis and neutrophil function by avridine in dexamethasone-treated and nontreated cattle. Am. J. Vet. Res. 46,53, 1985. 14. Roth, J.A. and Kaeberle, M.L. In vivo effect of ascorbic acid on neutrophil function in healthy and dexamethasone-treated cattle. Am. J. Vet. Res. 46,2434, 1985. 15. Roth, J.A., Kaeberle, M.L. and Griffith, R.W. Effects of bovine viral diarrhea virus infection on bovine polymorphonuclear leukocyte function. Am. J. Vet. Res. 42,244, 1981. 16. Roth, J.A., Kaeberle, M.L. and Hsu, W.H,. Effects of ACTH administration on bovine polymorphonuclear leukocyte function and lymphocyte blastogenesis. Am. J. Vet. Res. 43,412, 1982. 17. Roth, J.A., Kaeberle, M.L. and Hubbard, R.D. Attempts to use thiabenazole to improve the immune response in dexamethasone-treated or stressed cattle. Immunopharmacology 8,121, 1984. 18. Ryu, H., Kaeberle, M.L., Roth, J.A. and Griffith, R.W. Effect of type A *Pasteurella multocida* fractions on bovine polymorphonuclear leukocyte functions. Infect. Immun. 43,66, 1984. 19. Slauson, D.O. Clifford, C.B. and Holden-Stauffer, W. Alterations in membrane deformability and superoxide anion generation by neonatal calf neutrophils. J. Leukocyte Biol. 42,342, 1987. (Abstract) 20. Steinbeck, M.J. and Roth, J.A. Neutrophil activation by recombinant cytokines. Rev. Infect. Dis. 11,549, 1989. 21. Steinbeck, M.J., Roth, J.A. and Kaeberle, M.L. Activation of bovine neutrophils by recombinant interferon-gamma. Cell. Immunol. 98,137, 1986.