IMMUNOMODULATION: A MEANS OF DISEASE PREVENTION IN STRESSED LIVESTOCK

Frank Blecha

Kansas State University, Manhattan 66506

ABSTRACT

The ability to stimulate the immune response of cattle and pigs offers a new means of disease intervention. This review discusses current in vivo experiments that have evaluated immunomodulators in cattle and pigs. Levamisole, thiabendazole, imuthiol, avridine, isoprinosine, bovine recombinant interferon, human recombinant interleukin-2, bovine recombinant interleukin-2 and various supplemental vitamins and minerals have been used as immunomodulators in livestock with various degrees of success. Future research on immunomodulators, specific for domestic farm animals, will provide additional methods of treating immunosuppressed animals.

(Key Words: Immune Response, Immunity, Pigs, Cattle, Stress.)

Introduction

It is generally accepted that the culmination of production diseases in domestic food animals, such as respiratory disease in cattle and pigs, involves the interaction of host exposure to stressful stimuli with viral and bacterial challenges. Indeed, several studies have shown that stress- and viral-induced immunosuppression contributes to the pathogenesis of bovine respiratory disease (Blecha and Minocha, 1983; McGuire and Babiuk, 1983; Blecha et al., 1984; Filion et al., 1984; Roth, 1984; Babiuk and Ohmann, 1985). If stress- or viral-induced immunological defects in livestock can be related to problems in immune regulation, then methods of modulating the immune response should benefit calves and pigs. One approach to this problem is to administer substances exogenously that can augment the immune response and, hence, increase disease resistance.

Immunomodulators are substances of various origins that have the capability of regulating or modulating an immune response. As the term implies, immunomodulators may either augment or suppress an immune response. However, as will be the case in this review, the term immunomodulator is used often to refer to substances that augment immune responses. Other synonyms for immunomodulators include immunostimulants, immunopotentiators and biological response modifiers.

Recently extensive reviews have addressed the topic of stress and immune function in farm animals (Kelley, 1980; Kelley, 1982; Kelley, 1985; Siegel, 1985; Blecha, 1988). Several authors have compiled lists and chemical structures and have proposed modes of action of immunomodulators for use in domestic farm animals (Fenichel and Chirigos, 1984; Kende et al., 1984; Torrence, 1985, Mulcahy and Quinn, 1986). The purpose of this review is to discuss recent data on immunomodulators that have been used in vivo in cattle and pigs and to integrate our findings on immunomodulation in stressed livestock with these recent reports.

Levamisole and Thiabendazole

Levamisole and thiabendazole, two commonly used anthelmintics in cattle and pigs, also have been studied widely as immunomodulators. The immunostimulating capability of levamisole was reported first by Renoux and Renoux (1971). Since this first report, which showed an increased response to Brucella vaccination in mice treated with levamisole, several review
articles have discussed proposed mechanisms of action of the immunomodulatory properties of levamisole (Symoen and Rosenthal, 1977; Brunner and Muscoplat, 1980; Amery and Horig, 1984). Levamisole clearly is an immunopotentiating agent. However, the efficacy of levamisole in cattle and pigs is highly dependent on the condition of the animal (with most effectiveness in the stressed or immunocompromised host), on the dosage used and on the time of administration (Brunner and Muscoplat, 1980).

Levamisole administered (6 mg/kg) to unstressed calves at vaccination caused decreased antibody and cellular immune responses to bovine herpesvirus (Bibiuk and Misra, 1981). However, in a similar study using the same dose of levamisole in calves that were transported before treatment, an enhanced antibody response was observed (Babiuk and Misra, 1982). Levamisole has been found to decrease primary antibody responses and to enhance secondary antibody responses to sheep erythrocytes in pigs (Reyero et al., 1979) and to normalize the decreased cellular immune responses of artificially reared pigs (Hennessy et al., 1987).

Roth (1984) has evaluated several immunomodulators in cattle. A typical experimental protocol used by Roth and colleagues was to determine whether a potential immunomodulator could reverse the immunosuppressive effects of glucocorticoid administration. Levamisole administered at six different dosages (.5, 1, 2, 4 and 8 mg/kg body weight orally for 3 d and 6 mg/kg given for 1 d) did not normalize lymphocyte proliferative responses to mitogens in dexamethasone-treated calves (Roth and Kaeberle, 1984). Additionally, levamisole did not reverse the dexamethasone-induced suppression in neutrophil function or antibody-dependent cellular cytotoxicity. Alternatively, thiabendazole used in a similar dexamethasone-immunomodulator study did enhance mitogen-induced lymphocyte proliferative responses at dosages of 1, 3, 6, 12 and 25 mg/kg in dexamethasone-treated calves (Kaaberle and Roth, 1984). However, thiabendazole was not effective at 50 or 100 mg/kg and did not normalize neutrophil function or antibody-dependent cellular cytotoxicity in dexamethasone-treated calves. In a second study using thiabendazole (20 mg/kg for 5 d) in calves that had been weaned, castrated and placed in a feedlot, a decrease in antibody titers to a killed Brucella abortus vaccine was observed; no influence on antibody titers to equine ferritin or tetanus toxoid was found (Roth et al., 1984).

Flesh et al. (1982) have shown beneficial effects of levamisole (2.5 mg · mg⁻¹ · wk⁻¹ for 6 wk) in decreasing mastitis and fetal mortality in dairy cows. Similarly, Irwin et al. (1980) found a reduced incidence of bovine respiratory disease in feedlot cattle treated with levamisole phosphate (8 mg/kg) compared with calves treated with thiabendazole (66 mg/kg) or levamisole hydrochloride (8 mg/kg). However, untreated control animals were not included. In a study of transported feeder calves, we found reduced mortality in calves that were treated with levamisole upon arrival at the feedlot (6.8%) compared with calves injected with levamisole at an order-buyer barn prior to transport to the feedlot (9.0%) or with control calves that did not receive levamisole (13.6%) (Brazle et al., 1984).

Despite the fact that levamisole and thiabendazole occasionally have produced positive immunomodulating effects in cattle and pigs, their use as immunomodulators in stressed livestock probably is limited. The critical aspects of dosage and time of administration relative to stressful, immunosuppressive stimuli would appear to restrict their practical application.

Imuthiol

The inconsistent influence of levamisole on T-lymphocyte function has caused researchers to search for an agent devoid of inhibitory effects. Sodium diethyldithiocarbamate (imuthiol) is an immunomodulator that has been shown to have a marked stimulatory influence on T-lymphocyte function in vivo (Renoux and Renoux, 1984). It has been reported that imuthiol does not manifest the time- and dose-dependent characteristics that have limited the use of other immunomodulators, such as levamisole (Renoux, 1986). Immunosuppressed animals that were treated with imuthiol regained normal T-lymphocyte function (Bruley-Rosset and Renoux, 1985), and when imuthiol was administered to pregnant mice, T-lymphocyte-dependent responses in the offspring were enhanced along with neonatal growth rates (Renoux et al., 1985). The immunostimulating properties of imuthiol suggests to us that this compound might be an effective immunotherapeutic agent in weanling pigs.

In an experiment using 21, 3-wk-old pigs, we
evaluated the influence of imuthiol on T-lymphocyte function (Flaming et al., 1986). Pigs were injected subcutaneously with imuthiol at 2.5 or 25.0 mg/kg on the day of weaning; control pigs were injected with physiologic saline. Delayed-type hypersensitivity responses, mitogen-stimulated lymphocyte proliferative responses and interleukin-2 production were evaluated over an 18-d period. A single injection of imuthiol at 25.0 mg/kg reduced lymphocyte proliferative responses to concanavalin A and pokeweed mitogen. Imuthiol at 2.5 and 25.0 mg/kg lowered interleukin-2 production compared with saline-treated controls. Delayed-type hypersensitivity responses to phytohemagglutinin were increased in 25.0 mg/kg imuthiol-treated pigs, but were lower in 2.5 mg/kg imuthiol-treated animals. However, growth was reduced in both the 2.5 and 25.0 mg/kg imuthiol-treated pigs.

Most of the current literature suggests that imuthiol induces an increase in lymphocyte proliferative responses and interleukin-2 production (Renoux and Renoux, 1979; Renoux and Renoux, 1984; Chung et al., 1985); however, stimulation of lymphocyte blastogenesis has been reported to be delayed or to follow an initial period of suppression (Renoux and Renoux, 1984). Our data support previous reports showing that imuthiol had no, or a slightly inhibitory, effect on pokeweed mitogen-induced lymphocyte blastogenesis (Renoux and Renoux, 1984).

The reasons for the inhibitory effects of imuthiol in weanling pigs are unclear. The dosage of imuthiol that was used was consistent with published data for mice and humans (Renoux and Renoux, 1984); however, the correct dosage for a given drug often differs widely with species. Finally, reduction in growth observed in imuthiol-treated pigs suggests that even if the drug had produced immunostimulating effects in pigs its use in livestock might be limited.

Avridine

Avridine is a lipoidal amine (N, N-dioctadecyl- N', N'-bis[2-hydroxyethyl] propanediamine), previously referred to as CP 20,961, that has activity as an interferon inducer (Hoffman et al., 1973). Woodard et al. (1983) found that neutrophils from calves treated in vivo with liposome-encapsulated avridine (1.0 to 2.5 mg/kg) had increased bactericidal activity against Escherichia coli and Pasteurella multo-
cida. Roth and Kaeberle (1985a) conducted two experiments evaluating avridine in cattle. In the first experiment, liposome-encapsulated avridine was administered once at three different dosages (.1, 1.0 or 10.0 mg/kg) to Holstein steers. Avridine-treated calves had higher mitogen-induced lymphocyte proliferative responses than did control calves. Similarly, avridine treatment enhanced neutrophil phagocytosis of Staphylococcus aureus and antibody-mediated cellular cytotoxicity. However, the highest dose of avridine (10 mg/kg) decreased neutrophil iodination. The second study conducted by Roth and Kaeberle (1985b) evaluated the potential of avridine to reverse the immunosuppressive effects of dexamethasone. Avridine (10 mg/kg) given 24 h after dexamethasone administration (.04 mg/kg) prevented the suppressive effects of dexamethasone on neutrophil random migration, nitroblue tetrazolium reduction and antibody-dependent cellular cytotoxicity. Calves that were administered only dexamethasone did not show the characteristic depression in lymphocyte proliferative responses to mitogens or neutrophil ingestion of S. aureus. However, calves administered avridine plus dexamethasone had enhanced lymphocyte blastogenesis and neutrophil ingestion of S. aureus compared with either the dexamethasone-treated or control calves. The avridine treatment did produce an acute inflammatory response and caused a transient febrile response. Whether avridine treatment would reverse some of the immunosuppressive effects observed in stressed cattle has not been determined.

Isoprinosine

Compared with sow-reared littermate controls, artificially reared pigs had approximately 50% of the lymphocyte blastogenic response and a delayed-type hypersensitivity reaction to mitogens (Blecha et al., 1986). Therefore, we were interested in determining if an immunomodulator could reverse the immunosuppressive influence of artificial rearing in pigs. Levamisole and isoprinosine were the two immunomodulators that we used (Hennessy et al., 1987). The immunomodulatory capability of levamisole already has been discussed. Isoprinosine is an inosine-containing compound that has been investigated as an antiviral agent (Ginsberg and Glasky, 1977). However, it appears that the protective effect of isoprinosine is due, to large measure, to its immunomodulating and, in most
cases, immunopotentiating, action (O'Neill et al., 1984).

We used 30 pigs allotted to two groups, either sow-reared or artificially reared. Within these two main groups, pigs were allotted to either a control, a levamisole-treated or an isoprinosine-treated subgroup. Artificially reared pigs were removed from sows within 2 d after parturition and reared artificially for 21 d; sow-reared littersmates were kept with their dams. Levamisole (2 mg, s.c.) was administered on d 5 and 10. Isoprinosine (75 mg · kg$^{-1}$ · d$^{-1}$) was administered orally from d 0 to 10. Control pigs were given physiologic saline subcutaneously and orally. Control artificially reared pigs had suppressed lymphocyte blastogenic responses and delayed-type hypersensitivity reactions compared with sow-reared controls. However, both levamisole and isoprinosine enhanced the responses in the artificially reared pigs to values comparable to those of sow-reared controls. The amelioration of the immunosuppressive effect of artificial rearing by levamisole and isoprinosine stimulated us to investigate isoprinosine in other instances of immunosuppression in cattle and pigs.

Because stress- and viral-induced immunosuppression is strongly implicated in bovine respiratory disease, and because isoprinosine has a long history as an antiviral agent, we were very interested in evaluating isoprinosine in calves challenged with bovine herpesvirus type-1 (BHV-1; Blecha et al., 1987b). Twenty-four calves were allotted to two groups: control calves and calves that were challenged with BHV-1. Within each of these groups, six calves received isoprinosine (75 mg · kg$^{-1}$ · d$^{-1}$, orally) for 14 d and six control calves received a placebo. Bovine herpesvirus-infected calves had increased rectal temperatures at 3 to 7 d post-infection; isoprinosine did not reverse this effect. Additionally, phytohemagglutinin-induced lymphocyte proliferative responses were lower in BHV-1 infected calves compared with control or isoprinosine-treated calves on d 4 postinfection. Isoprinosine did not reverse this BHV-1 induced suppression in lymphocyte proliferation. We also have conducted a similar experiment evaluating isoprinosine in pseudorabies virus-infected pigs (Flaming et al., 1987). Unfortunately, isoprinosine did not ameliorate the suppressive effect of pseudorabies virus infection on porcine lymphocyte function.

The reason we observed a beneficial effect of isoprinosine in artificially reared pigs and not in herpesvirus-infected cattle or pigs is not clear. However, the difference in response suggests that immunomodulators that are effective in reversing the immunosuppressive effects of environmental or management stressors may not produce the same results in viral-induced immunosuppression.

**Cytokines**

Similar to other biological systems, proteins produced by cells of the immune system interact with cell receptors and are critical in regulating or modifying immune reactivity. Cytokines such as the interferons, interleukins and colony-stimulating factors are available now through recombinant DNA technology in sufficient amount to evaluate their in vivo efficacy in livestock.

Calves treated with recombinant bovine interferon (rBoIFN$\alpha$) prior to challenge with BHV-1 had an enhanced ability to withstand a subsequent Pasteurella haemolytica challenge (Babiuk et al., 1985). The interferon treatment did not appear to have a direct effect on viral replication, because viral shedding from the nasal passages did not differ between the control and interferon-treated calves. However, the interferon-treated calves had a lower incidence of fibrinous pneumonia than did the control calves, suggesting that rBoIFN$\alpha$ may have a greater immunomodulatory effect than a direct antiviral effect. These same researchers (Bielefeldt-Ohmann et al., 1986) have shown that rBoIFN$\gamma$, and to a lesser degree rBoIFN$\alpha$, given in vivo to calves increased the number of alveolar macrophages that were positive for the bovine Ia antigen. Because Ia antigens are important in antigen presentation to T cells and antigen-specific T cell proliferation (Unane, 1980), interferon-induced expression of Ia antigens on alveolar macrophages is an extremely important immunomodulatory function of interferon.

The lymphokine interleukin-2 (IL-2) is released by stimulated T-cells and is required for clonal expansion of T-lymphocytes (Gillis and Smith, 1977). Defects in production and responsiveness to IL-2 have been found in many immunodeficiency states (Thoman and Weigle, 1982; Linker-Israeli et al., 1983; Reiner and Finke, 1983). We have shown that physiologically attainable high levels of cortisol lower IL-2 production in vitro and in vivo in cattle and pigs (Blecha and Baker, 1986; Klemcke et al., 1987). Babiuk and Ohmann (1985) have found
that BHV-1-induced immunosuppression decreases bovine IL-2 production. These findings imply that in vivo administration of IL-2 to livestock may be beneficial to combating stress- or viral-induced immunosuppression.

In vivo administration of human recombinant interleukin-2 (rIL-2) enhanced the immune response in pigs vaccinated and challenged with Haemophilus pleuropneumoniae (Fedorka-Cray et al., 1986; Urban et al., 1986). Pigs administered human rIL-2 had higher antibody titers to H. pleuropneumoniae and a lower incidence of lung lesions. We have conducted an experiment to determine the in vivo efficacy of bovine rIL-2 in feeder calves vaccinated and then challenged with BHV-1 (Blecha et al., 1987a). Serum neutralizing antibody titers to BHV-1 were increased sixfold in calves vaccinated and treated with bovine rIL-2 compared with calves that were vaccinated only. Bovine rIL-2 treatment induced lymphokine-activated killer cells in calves independent of BHV-1 vaccination. Calves vaccinated and treated with bovine rIL-2 had improved clinical signs after challenge with BHV-1 compared with calves that were vaccinated but did not receive bovine rIL-2. However, the dose of bovine rIL-2 that was used caused significant illness in the calves that abated after rIL-2 treatment was discontinued. Despite the problems that were encountered with the adverse side effects of rIL-2 administration, the enhancement in antibody titers, increased cytotoxic responses and enhanced ability to withstand a BHV-1 challenge warrant more intensive evaluation of bovine rIL-2 dosages in calves.

Vitamins and Minerals

The association between nutrition and disease resistance is well recognized. However, this association is not limited just to severe or moderate deficiency states. Therapeutic uses of vitamins and minerals well above recommended requirements are being evaluated for modulation of immune reactivity (Watson, 1984). A discussion of the influence of dietary constituents on immune function is beyond the scope of this review. However, various supplementary vitamins and minerals given to cattle and pigs have been shown to influence immune reactivity. Roth and Kaeberle (1985b) found that vitamin C injected (20 mg/kg) into cattle increased neutrophil oxidative metabolism and neutrophil-mediated, antibody-dependent cellular cytotoxicity. Additionally, vitamin C tended to reverse the immunosuppressive effects of dexamethasone administration on neutrophil random migration, oxidative metabolism and antibody-dependent cellular cytotoxicity. However, Yen and Pond (1987) and Kornegay et al. (1986) found that vitamin C supplementation had no influence on porcine cellular and humoral immune responses. Peplowski et al. (1981) found that dietary and injectable vitamin E and Se enhanced antibody titers to sheep erythrocytes. Blodgett et al. (1986) observed a tendency for an increased humoral immune response in Se-supplemented pigs. However, delayed-type hypersensitivity reactions to phytohemagglutinin were not influenced by Se supplementation.

Other nutrients have been found to influence various immune parameters when supplemented above recommended dietary levels. However, in most instances, in vivo experiments evaluating the influence of vitamins and minerals, other than vitamins C and E and Se, on immune function in cattle and pigs have been limited.

Conclusions

There are long lists of substances that have been shown to have immunomodulatory capabilities in mammals (Kende et al., 1984). Some of these substances, such as the muramyl peptides, have shown promise in vitro in potentiating porcine immune responses (Charley et al., 1983). However, ultimately, the final test of a potential immunomodulator must be its in vivo efficacy. An additional requirement of immunomodulators used in cattle and pigs is that they not adversely influence growth performance. Because many immunomodulators are developed originally for human chemotherapeutic application, for which nausea and malaise are a small price to pay for enhanced immune function, this criterion is not typically appraised. However, future research on specific immunomodulators with minimal side effects for livestock will provide a new means of disease intervention in cattle and pigs.

Literature Cited

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Chung, V., I. Florentin and G. Renoux. 1985. Effect of imuthiol administration to normal or imuno-deficient mice on IL 1 and IL 2 production and immune responses regulated by these mediators. Int. J. Immunopharmacol. 7:335.


Kornegay, E. T., J. B. Meldrum, G. Schurig, M. D.


