Immunosuppressive Effects of Bovine Viral Pathogens: A Review

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ABBREVIATIONS USED: ADCC: antibody dependent cellular cytotoxicity, BAM: bovine alveolar macrophage, BHV1: Bovine Herpesvirus type 1, BLV: Bovine Leukemia Virus, BRDC: bovine respiratory disease complex, BRSV: Bovine Respiratory Syncytial Virus, BTV: Blue Tongue Virus, BVDV: Bovine Viral Diarrhea Virus, ConA: Concanavalin A, CPE: cytophathic effect, Ig: immunoglobulin, PHA: phytohemagglutinin, PI3: Bovine Parainfluenza Virus type 3.

Introduction

The interactions of the various factors that lead to a diseased state in an animal are complex and incompletely understood These interrelated factors may be placed into three simultaneously interacting groups: host factors, parasite factors, and extra-host/extra-parasite factors (Table 1). Host factors include both specific and nonspecific host defense mechanisms. Additionally, the age, genetic makeup, and the nutritional, physiological, and psychological status of the animal are important as they affect these host factors. The critical parasite factors are those that determine virulence and/or pathogenicity. Finally, there are factors not directly associated with either the host or the parasite that affect them both and their interaction. These include environmental conditions and the effects of other pathogens.

There are many examples of some or all of these factors contributing to disease. One example is found in cattle, where host factors, parasite factors, environmental factors, and primary and secondary pathogens all interact to cause BRDC. Examples of this type of phenomenon in cattle are not limited to pneumonic disease.

Primary viral infections may cause clinical disease on their own depending on the type of virus and condition of the host. More importantly, they frequently predispose an animal to secondary complications such as bacterial infections. This is especially true when viral effects are combined with other immunosuppressive factors such as stress, poor nutrition, and concurrent diseases. Viral infections interact in this complex system by their detrimental effects on host defense mechanisms. This paper will review the immunosuppressive effects on cattle attributed to various bovine viral pathogens.

Bovine Herpesvirus Type 1

This virus, also known as Infectious Bovine Rhinotracheitis Virus, has been extensively studied because of its important role in BRDC. Clinically, BHV1 may manifest itself as upper respiratory tract infection, conjunctivitis, infectious pustular vulvovaginitis, abortion, and nonsuppurative meningoencephalitis.

TABLE 1.

HOST FACTORS
Normal Flora
Physical Barriers
(skin, mucous membrane, cornea,
mucociliary apparatus, tears)
Antimicrobial Components
(complement, lysozyme, transferrin, stomach acid,
beta-lysin)
Phagocytes
(macrophages and polymorphonuclear neutrophils)
Cell-mediated and Humoral Immunity
PATHOGEN FACTORS
Capsule
Leukocidins
Invasins
Adhesins
Motility
Toxins
Iron Transport Mechanisms
Serum Resistance
Drug Resistance
Antigenic Changes
Tolerance
Immuts
nonsuppression
NON-HOSTS/NON-PATHOGEN FACTORS
Environment
Infection by Concurrent Pathogens

BHV1 has been shown to have detrimental effects on the bacterial defense mechanisms of the lung. Aerosol exposure to BHV1 facilitated infection of the lung by a usually noninfectious doses of *P. hemolytica* resulting in fibrinous pneumonia.[1-6] Additionally, after calves exposed to *P. hemolytica* serotype 1 had apparently cleared the bacteria from their nasal passages, intranasal inoculation with BHV1 caused recrudescence of *P. hemolytica* in two of eight calves.[7] *In vitro* and *in vivo* experiments have shown CPE on respiratory epithelium that results in compromise of the mucociliary defense mechanisms.[8-9]

Altered BAM functions have also been attributed to BHV1. BHV1 has been reported to have CPE on BAM,[10,11] even though it appears to replicate in BAM at very low levels[11-13] or not at all.[10] Other BHV1 mediated effects noted in these *in vitro* BAM studies include reduction of Fc-mediated receptor activity and phagocytosis, complement receptor activity, and ADCC.[13]

In vivo BHV1 infection also affects BAM recovered by lung lavage. One study reported that only a small proportion of BAM appeared to become infected in aerosol or intranasal challenged calves.[14] In this study even though clinical signs of viral infection were noted, the BAM from these calves showed unaltered Fc and complement receptor activities, phagocytic activity, and ability to mediate ADCC.[14] Macrophage activity as measured by chemiluminescence is also impaired in BHV1 infected calves (P. D. Conlon, and P. Eyre, Abstr. Annu. Meet. Conf. Res. Workers in Animal Dis. 1985, 79, p. 15). In another study of aerosol challenged calves, lavage recovered BAM showed increases in the percentage of cells expressing a major histocompatibility class II antigen, increased Fc-mediated phagocytosis, increased beta-glucuronidase, and increased production to prostaglandin E2.[15] Selective suppression of BAM mediated cellular cytotoxicity and interleukin-1 generation was observed in this study.[15] In an experiment using an aerosol BHV1 challenge the percentage of BAM with Fc and C3b receptors was significantly reduced six days post-exposure.[16] These BAM had significantly reduced ability to phagocytize opsonized *Staph. epidermidis* but were able to kill ingested bacteria.

Macrophage/neutrophil interaction, as well as neutrophil function, may also be impaired in calves exposed to an aerosol of BHV1 virus followed five days later by an aerosol of *P. hemolytica*.[17] Analysis of sequential lavage fluids suggested that neutrophil infiltration into the lung in response to the presence of the bacteria was delayed. *In vitro* studies of these cells showed that neutrophils from infected animals displayed little random migration and did not respond to a chemotactic stimulus.[17] Macrophages were not able to produce neutrophil chemotactic factors.[17]

In another BHV1 aerosol challenge study, significant depression of neutrophil chemotactic response was noted, but the antibacterial activity of the neutrophils was not significantly affected.[18] In calves inoculated with an aerosol of BHV1 there was enhancement in *Staph. aureus* ingestion by neutrophils during post-exposure days 4 through 11.[19] BHV1 infection did not significantly affect cytochrome-c reduction, ADCC, chemiluminescence, or iodination. In an *in vitro* study of the direct effects of BHV1 on the interaction of blood neutrophils with *P. hemolytica* A1, neutrophils were incubated with BHV1. No effects were noted on neutrophil random or directed migration, ability to ingest and kill *P. hemolytica* A1, chemiluminescence, or susceptibility to the lethal effects of crude *P. hemolytica* cytotoxin.[20]

A study examining lymphocyte function demonstrated a significant depression in the lymphocyte blastogenic response to PHA, *P. hemolytica*, and *P. multocida*.[18] *In vitro* infection of lymphocytes with live BHV1 inhibited blastogenic response of bovine peripheral blood mononuclear cells to PHA while infection with ultraviolet irradiated BHV1 caused no effects (A. Pollard, N.S. Magnuson, T. Yilma, R. Reeves, and J.A. Magnuson, Fed. Proc. 44:529, 1986). Intravenous inoculation of BHV1 decreased the number of circulating lymphocytes by more than 50%.[21]

Bovine Parainfluenza Virus Type 3

Infection with this virus results in mild to moderate respiratory disease. Infection has been shown to predispose cattle to the development of bacterial pneumonia following aerosol exposure to *P. hemolytica*[22-24] or *P. multocida*[25,26] Aerosol exposure of calves to PI3 followed by aerosol exposure to *P. hemolytica* results in reduced clearance of *P. hemolytica*.[27-29] In one study, reduced clearance was noted on the first day following PI3 exposure but noted on any of the subsequent 14 days.[27] In another experiment, reduced clearance was not on days 7 and 11 but not on day three.[28] Finally, one study showed no suppressive effect on pulmonary clearance in six out of seven calves, seven days after PI3 exposure.[29] Additionally, after calves exposed to *P. hemolytica* serotype 1 had apparently cleared the bacteria from their nasal passages, intranasal inoculation with PI3 caused recrudescence of *P. hemolytica* in 6 of 20 calves.[7] Like BHV1, PI3 infection readily destroyed the ciliary activity in tracheal ring cultures.[10]

BAM cultures infected *in vitro* with PI3 show CPE.[10,11] In one study, infective virions were released into the media.[11] In another, the virus failed to survive five passages and thus did not meet the author's criteria for replication in macrophages.[10] PI3 replicated and caused CPE in another *in vitro* study on cultured BAM.[30] Additionally, infected BAM showed depressed phagocytic activity against *Candida glabrata* and a marked reduction in phagosome/lysosome fusion.[30] Similarly, PI3 infected cultures of BAM were less adherent to glass, less efficient at phagocytizing antibody coated sheep erythrocytes, had significantly lower concentrations of acid phosphatase, and showed decreased bacterial killing.[31] Significant differences in latex particle phagocytosis were not noted

between infected and control cultures.[31] Interestingly, when the PI3 virus infected BAM were exposed to antiserum of to Ig against PI3 virus there was an approximately 2-fold greater inhibition of antibody coated sheep erythrocyte phagocytosis when compared to infected BAM exposed to serum without anti-PI3 activity.[31] In a study of calf BAM cytotoxicity activity, cells washed from lungs of freshly killed calves were cytotoxic for calf kidney target cells infected with PI3 virus.[32] This cytotoxic reaction was inhibited by PI3 antiserum. When BAM were added to PI3 infected cell cultures the yield of virus was reduced for the first two to three days. Subsequently, virus replicated in the BAM reducing their cytotoxic activity.[32]

BAM harvested from cattle infected with PI3 also show compromised function. In an experiment using an aerosol PI3 challenge, the percentage of BAM with Fc and C3b receptors was significantly reduced six days post-exposure.[16] These BAM had significantly reduced ability to phagocytize opsonized *Staph. epidermidis* but were able to kill ingested bacteria. In another study, PI3 infection of calves significantly impaired alveolar macrophage chemiluminescence and beta-adrenoceptor function (P.O. Ogunbiyi and P. Eyre, Fed. Proc. 44:469, p. 491, 1986). The phagocytic kinetics of BAM were examined in calves seven days following an intranasal aerosol exposure to PI3. In virus-infected animals a decreased proportion of BAM were phagocytic and the mean initial phagocytic rates of BAM were significantly decreased.[33]

Chemiluminescence and iodination ability of neutrophils were significantly reduced in calves aerosol exposed to PI3. Random migration, *Staph. aureus* ingestion, cytochrome-c reduction, and ADCC were not significantly affected.[19]

Lymphocytes from calves experimentally infected intratracheally with PI3 showed reduced response to stimulation with PHA as determined by reduced 3H-thymidine uptake in blastogenesis assays. The effects were most severe between the days two to seven and gradually abated from days eight to ten.[34]

Bovine Respiratory Syncytial Virus

BRSV was reported to be associated with acute respiratory disease in calves in 1970.[35] Spontaneous and experimentally induced infection in calves results in bronchitis, bronchiolitis, interstitial pneumonia, and other respiratory tract lesions[36,37] and may predispose to secondary bacterial pneumonia.[38,39]

BRSV inoculated into *in vitro* tracheal ring cultures was able to replicate within the ciliated epithelial cells of the tracheal rings, yet ciliary activity was not destroyed.[10] In contrast, electron microscopy of one-month-old calves infected with BRSV showed that viral assembly and release in tracheal and bronchial epithelial cells was associated with loss of cilia.[40] It was noted that regeneration of the airway epithelium was largely completed by 10 days after inoculation. One calf had failure of epithelial repair and developed secondary bacterial pneumonia.[40] Experimental inoculation with BRSV produces a mild leukopenia.[41]

Compared to other bovine respiratory viruses, BRSV is not as infective for BAM. In one study of BRSV infected BAM cultures, no CPE was noted, a small number of BRSV virions were consistently released, and about one percent of the macrophages were fluorescent antibody-positive for viral antigen.[11] Others have confirmed the lack of CPE but have failed to demonstrate viral replication in BAM.[10,42]

In vitro inoculation of BAM with BRSV impairs selected functions.[42] Fc-mediated phagocytosis of antibody coated sheep erythrocytes was significantly impaired in BRSV infected cultures, but no differences were noted in the macrophage viability, ability to adhere to glass, killing ability, latex bead phagocytosis, or lysosomal enzyme content.[42]

Bovine Viral Diarrhea Virus

BVDV produces different clinical syndromes depending on immunological status and presence or absence of infection. Cattle that are persistently infected with BVDV and fail to produce an antibody response are susceptible to a fatal condition termed mucosal disease.[43] Experimental infection of healthy cattle with BVDV usually results in a transient febrile response and leukopenia with clinical signs such as depression, anorexia, and tachypnea.[44] Like many other bovine viruses, much of the importance of BVDV infection abides in its immunosuppressive effect, facilitating the occurrence of secondary infections.

There are reports of an increased incidence of bacterial infection in calves recently infected with, or seroconverting to BVDV.[45] As was previously mentioned for other bovine viruses, BVDV will facilitate pneumonic infection with a usually noninfectious does of *P. hemolytica* under the appropriate experimental conditions.[46] BVDV readily destroyed the ciliary activity in infected cultures of bovine tracheal rings.[10] One study reports that BVDV had no statistically significant effect on pulmonary clearance of *P. hemolytica*.[47] In their discussion the authors attribute this to the great variation within and between animals of the same experimental group.

An experiment was conducted in which six cattle persistently infected with and seronegative to BVDV were inoculated with lymphocytes infected with BLV. While all controls seroconverted to BLV four to five weeks after inoculation, four of the six BVDV infected cattle had delayed, weak antibody responses. BLV was isolated from all the cattle persistently infected with BVDV at 42 or 58 weeks after BLV inoculation regardless of whether the serum samples gave negative, weak, or positive BLV results, suggesting decreased immune responsiveness to BLV in animals persistently infected with BVDV.[48]

In vitro studies on BAM indicate that BVDV is capable of replicating and causing CPE.[10,11] In one of the studies the more virulent strains were better able to replicate in BAM.[10] Another study using peripheral blood monocytes showed that incubation with BVDV caused decreases in random locomotion and chemotaxis.[49] These effects were not noted when monocytes were incubated with heat killed BVDV.

BVDV also affects neutrophils. A modified live vaccine strain of BVDV administered intranasally or intramuscularly to cattle produced a decrease in the number of circulating neutrophils, as well as suppression of iodination and neutrophil mediated ADCC.[50] No statistically significant changes occurred in other neutrophil parameters, including random migration under agarose, ingestion of *Staph. aureus*, and nitroblue tetrazolium reduction.[50] In another experiment, *in vivo* infections with cytopathogenic and noncytopathogenic BVDV caused a marked impairment of iodination, but paraffin oil uptake, nitroblue tetrazolium reduction, and chemiluminescence were not decreased.[51] Neutrophils from cattle persistently infected with BVDV had a significantly impaired ability to ingest *Staph. aureus* but were normal in respect to random migration under agarose, cytochrome C reduction, iodination, and ADCC.[52] However, a case report of a bull persistently infected with BVDV stated that neutrophils had a greater ability to ingest *Staph. aureus* and significantly reduced ability to iodinate protein.[53] In one of these reports the authors noted that impairment of neutrophil function in cattle persistently infected with BVDV differs from impairment of neutrophil function reported in healthy cattle mounting an immune response to recent BVDV infection.[52]

A variety of lymphocyte suppressive phenomenon have been associated with BVDV. Administration of a modified live vaccine strain of BVDV resulted in a decrease in the number of circulating lymphocytes and a decrease in their ability to respond to mitogens.[50] In cattle persistently infected with BVDV, uptake of ^{3}H-thymidine by both resting and mitogen stimulated peripheral blood mononuclear cells was suppressed.[52-54] Inoculation of healthy cattle with a cytopathic strain of BVDV produced a similar drop in circulating lymphocytes and neutrophils, as well as a drop in the percentage and absolute number of T lymphocytes.[55] The absolute number of B lymphocytes also decreased but there was no apparent detrimental effect on antibody response to viral antigen.[55] It was also noted that the percentage and absolute number of cells that could not be identified as either B or T lymphocytes increased following viral challenge.[55] When calves were experimentally inoculated with low doses of cytopathic and noncytopathic BVDV no differences were noted between infected cattle and controls for PHA stimulated blastogenesis, percentage of lymphocytes with surface Ig, and antibody titers induced by an intravenous inoculation of killed *Brucella abortus*.[56] The authors refer to differences in the strains of virus used and the handling prior to inoculation that may account for these results. In cattle affected by fatal mucosal disease, serum levels of IgG2 were significantly reduced.[43] Sera from 6 of 12 animals in later stages of fatal mucosal disease had a marked suppressive effect on lymphocyte blastogenesis of normal lymphocytes.[43] Washed lymphocytes from sick animals responded similarly to control lymphocytes when stimulated with PHA and ConA.[43] Two cattle chronically infected with BVDV were reported to have reduced numbers of surface Ig positive cells, B lymphocytes, and other abnormalities that the authors interpret as indicative of B cell dysfunction.[57]

In vitro studies of lymphocytes have also shown suppressed function in the presence of BVDV. Blastogenic responses of bovine lymphocytes to PHA were depressed when BVDV was added, however, no depression occurred if ultraviolet irradiated BVDV was added.[58] Bovine fetal lung tissue cultures infected with BVDV release into the supernatant fluid low molecular weight substances which suppress the proliferative response of bovine peripheral blood mononuclear cells to conA.[59]

Bovine Leukemia Virus

In the adult form of bovine leukosis, infection of cattle with BLV consistently results in seroconversion[60] and one of four clinical conditions: 1) inapparent carrier state with normal lymphocyte number and normal B and T lymphocyte ratio, 2) normal lymphocyte number and inverted B and T lymphocyte ratio, 3) peripheral lymphocytosis composed of predominantly B lymphocytes,[61] or 4) lymphosarcoma.[62,63] The most common condition is the inapparent carrier. Peripheral lymphocytosis occurs in approximately 30% of the BLV seropositive cattle and lymphosarcoma occurs in less than 1% of the BLV seropositive cattle.[64] Three rarer forms of bovine leukemia are recognized: thymic form, juvenile or calf form, and the cutaneous of skin form.[65]

In epidemiologic studies of cows with and without antibodies to BLV, cull rates increased significantly as BLV positive cows aged, but not as antibody negative cows aged.[66] The authors speculated on the possible association between BLV, morbidity and mortality rates, and possible BLV induced immunosuppression.

In leukemic cattle, antibodies to various antigens appeared after an interval twice as long as in normal cattle and were produced either in low or undetectable amounts.[67] The leukemic cattle also exhibited an inconsistent IgM/IgG ratio in their sera. The authors concluded that the immune response in leukemic cows was either depressed or B cells were producing incompetent Ig molecules.[67] When peripheral blood lymphocytes from BLV infected cattle with persistent lymphocytosis or leukemia were compared to normal cattle, the cytotoxic activity in infected animals was markedly decreased.[68]

In a study comparing skin allograft rejection, lymphocyte blastogenesis, immunoglobulin quantities, antibody

titers to BLV and BHV1, and antibody response to immunization with chicken red blood cells, no differences were noted between healthy cattle and cattle with leukosis.[69] However, another study showed that most sera from leukemic cattle inhibited phagocytic activity of normal bovine blood neutrophils, growth of interleukin 2-dependent bovine T cells, and mitogen induced blastogenesis of normal bovine lymphocytes, while ADCC and spontaneous cell-mediated cytotoxicity were suppressed by only a few sera.[70] The antibody titer against BLV in the sera correlated with the percent inhibition of lymphocyte blastogenesis.

Studies of RNA synthesis of blood lymphocytes showed higher spontaneous RNA synthesis in cells from BLV-infected cows with persistent lymphocytosis compared to normal cows.[71] There were no differences in RNA synthesis in response to mitogen stimulation in lymphocytes from infected and noninfected cows. Another report noted high levels of spontaneous lymphocyte blastogenesis in BLV-infected cattle, however lymphocyte responsiveness to mitogens was comparable to BLV-negative animals.[72]

Bluetongue Virus

Bluetongue virus infection of cattle results in laminitis and mucosal ulcers.[73] The immunosuppressive effects of BTV have been virtually unstudied. In one report BAM were exposed to BTV *in vitro*.[10] BTV was initially cytopathic. It lost its cytopathogenicity on repeated passage although it was capable of continued replication in macrophages.[10] Results of another study indicate that bovine monocytes are readily infected *in vitro* with BTV serotype 10.[74] In bovine fetuses BTV infection did not consistently suppress mitogen induced lymphocyte blastogenesis.[75]

Bovine Rotavirus and Coronavirus

These two viruses are considered primary etiologic agents of neonatal calf diarrhea. Electron micrographs of affected intestinal epithelium show severe compromise of this physical barrier.[76,77] Some authors have speculated on the role of these ciruses in predisposing calves to other infectious agents,[78] but no information concerning their effects on macrophage, neutrophil, or lymphocyte function is available.

Summary

Epidemiologic evidence that viral immunosuppression leads to secondary bacterial pneumonia is established, yet our understanding of the immunosuppressive viral effects that cause this is incomplete. Similar relationships are likely to exist in other disease complexes. This paper reviewed many of the effects of different bovine viral pathogens on the bovine host defenses such as epithelial barriers and ciliary function, macrophages, polymorphonuclear neutrophils, and lymphocytes. For each virus there are voids in our understanding. There are many viruses such as bovine adenovirus, bovine enterovirus, bovine empheral fever virus, bovine papilloma virus, bovine papular stomatitis virus, and bovine parvovirus for which no information is available. The relevance of current *in vitro* tests to *in vivo* function is, in many cases, speculative. There are many aspects of the host immune system, such as cell-mediated immunity, for which tests are insufficient or entirely lacking. Continued work is needed to develop means to examine the immunologic interactions of cattle and their viral pathogens. There is currently a great deal of research examining the effects of various immunomodulators in cattle. In order to effectively use these immunomodulators the nature of the immunosuppression that viruses cause must be further characterized.

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