Bovine Herpesvirus Type 1 (BHV-1) is an Important Cofactor in the Bovine Respiratory Disease Complex

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Infection of cattle by bovine herpesvirus 1 (BHV-1) can lead to upper respiratory tract disorders, conjunctivitis, genital disorders, and immune suppression. BHV-1–induced immune suppression initiates bovine respiratory disease complex (BRDC), which costs the US cattle industry more that a billion dollars each year. In addition, BHV-1 is an emerging virus in buffalo. The ability of BHV-1 to inhibit immune responses is crucial for the ability of BHV-1 to induce BRDC. BHV-1 encodes at least 3 proteins that can inhibit specific arms of the immune system: (1) the UL49.5 protein, (2) bICP0, and (3) glycoprotein G. Furthermore, BHV-1 can infect and induce high levels of apoptosis of CD4\textsuperscript{+} T cells, which also inhibit an efficient immune response. Following acute infection, BHV-1 establishes latency in sensory neurons of trigeminal ganglia (TG), and germinal centers of pharyngeal tonsil. Periodically BHV-1 reactivates from latency, virus is shed, and consequently virus transmission occurs. The latency-related gene is abundantly expressed in sensory neurons during latency and expression of a protein encoded by the latency-related gene is necessary for the...
The ability of BHV-1 to enter permissive cells, infect sensory neurons, and promote virus spread from sensory neurons to mucosal surfaces following reactivation from latency is also regulated by several viral glycoproteins. BHV-1 modified live vaccines can be immunosuppressive as well as establish and reactivate from latency, which indicates that these strains can lead to BRDC in feedlots or cow-calf operations. This review summarizes the role that BHV-1 plays in BRDC.

DISEASE AND CLINICAL SIGNS INDUCED BY BHV-1

Bovine herpesvirus 1 (BHV-1) is an α-herpesvirinae subfamily member that causes significant economic losses to the cattle industry. Three BHV-1 subtypes, BHV-1.1 (1), BHV-1.2a (2a), and BHV-1.2b (2b), have been identified based on antigenic and genomic analysis. Subtype 1 virus isolates are the causative agent of infectious bovine rhinotracheitis (IBR), and are frequently found in the respiratory tract as well as in aborted fetuses. Subtype 1 strains are prevalent in Europe, North America, and South America. Subtype 2a is frequently associated with a broad range of clinical manifestations in the respiratory and genital tracts, such as IBR, infectious pustular vulvovaginitis (IPV), balanopostitis (IPB), and abortions. Subtype 2a is prevalent in Brazil, and was present in Europe before the 1970s. Subtype 2b strains are associated with respiratory disease and IPV/IPB, but not abortion. Subtype 2b strains are less pathogenic than subtype 1, and are frequently isolated in Australia or Europe, but not Brazil.

In feedlot cattle, the respiratory form of BHV-1 is the most common (subtype 1 strains). In breeding cattle, abortions or genital infections tend to be more common. Genital infections can occur in bulls (IPB) and cows (IPV) within 1 to 3 days of mating or close contact with an infected animal. Transmission can also occur in the absence of visible lesions and through artificial insemination with semen from subclinically infected bulls.

The incubation period for the respiratory and genital forms of BHV-1 is 2 to 6 days. Clinical signs of respiratory disease include high fever, anorexia, coughing, excessive salivation, nasal discharge, and conjunctivitis with lacrimal discharge, inflamed nares, and dyspneae if the larynx becomes occluded with purulent material. Nasal lesions consist of numerous clusters of grayish necrotic foci on the mucous membrane of septal mucosa. In the absence of bacterial pneumonia, recovery typically occurs 4 to 5 days after the onset of clinical signs. Abortions can occur at the same time as respiratory disease, but are also seen up to 100 days after infection, presumably the result of reactivation from latency.

With respect to genital infections, the first clinical signs are frequent urination, and a mild vaginal infection. It is also common to observe swollen vulva or small papules followed by erosions and ulcers on the mucosal surface. In bulls, similar lesions occur on the penis and prepuce. If secondary bacterial infections occur, there may be inflammation of the uterus and transient infertility with purulent vaginal discharge for several weeks. In the absence of bacterial infections, animals usually recover within 2 weeks after infection. Regardless of the involvement of secondary bacterial infection, BHV-1 establishes lifelong latency following acute infection. Serologic testing and removal of infected animals has been used to eliminate BHV-1 from Austria, Denmark, and Switzerland. In these countries, cattle populations are small and movement of cattle can be controlled. In the United States and many other countries, eradication will be difficult, perhaps impossible, and expensive.
BHV-1 IS AN EMERGING DISEASE IN BUFFALO

The seroprevalence of BHV-1 in bison raised on a ranch is 43.8%, indicating that infection can readily occur in bison. Other studies have also found that BHV-1 is frequently present in buffalo in India. BHV-1 can presumably be transmitted from buffalo to cattle and vice versa. Because more buffalo meat is being consumed each year in the United States and other nations, BHV-1 infections are having an effect on the emerging bison industry. It is not clear what the percentage of seropositive animals is due to vaccination or infection with virulent field strains. It is also not well established whether BHV-1 can be readily reactivated from latency in buffalo, as in cattle. It is clear that BHV-1 is widely disseminated in buffalo herds, suggesting that BHV-1 could induce BRDC in buffalo.

RELATIONSHIP BETWEEN BHV-1 AND BRDC

BRDC, also known as shipping fever, costs the US cattle industry at least $1 billion/year. In addition to the clinical signs described earlier for BHV-1, BHV-1 can initiate BRDC by transiently suppressing the immune system of infected cattle. BHV-1–induced immune suppression leads to secondary bacterial infections (eg, Pasteurella haemolytica, Pasteurella multocida, and Histophilus somni) that can cause pneumonia. Increased susceptibility to secondary infection correlates with depressed cell-mediated immunity after BHV-1 infection. CD8+ T cell recognition of infected cells is impaired by repressing expression of major histocompatibility complex class I and the transporter associated with antigen presentation. CD4+ T cell function is impaired during acute infection of calves because BHV-1 infects CD4+ T cells and induces apoptosis. Viral genes (UL49.5, bICP0, and gG) can inhibit specific immune responses in the absence of other viral genes. The ability of bICP0 to inhibit interferon (IFN)-dependent transcription is crucial for pathogenesis because BHV-1 does not grow in mice unless they lack IFN receptors. The known viral genes that inhibit immune responses are discussed later.

UL49.5

The BHV-1 gene encoding the UL49.5 open reading frame (ORF), also known as glycoprotein N (gN), is present in the unique long region of the genome (Fig. 1). The UL49.5 ORF encodes a 96 amino acid protein with an apparent molecular mass of

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**Fig. 1.** Specific genes within the BHV-1 genome, showing the location of viral genes within the genome (bICP0, bICP4, gG, gE, Us9, UL49.5, UL10, and the latency related [LR] gene). The unique long (L) and unique short (S) regions of the genome are shown. The repeats are denoted by open rectangles. The bICP0 and bICP4 genes are in the repeats, and are thus present in 2 copies within the BHV-1 genome.
The UL49.5 ORF contains a signal peptide (N terminal 22 amino acids), an extracellular domain of 32 amino acids, a transmembrane region of 25 amino acids, and a 17-amino-acid cytoplasmic tail. The UL49.5 protein is expressed as a nonglycosylated type I membrane protein.

All gN herpesvirus orthologs studied to date form complexes with glycoprotein M (gM), which is encoded by the UL10 ORF (see Fig. 1 for location of gM). gM is important for secondary envelopment because, in pseudorabies virus (PRV), secondary envelopment in the Golgi and subsequent egress requires gM, in the absence of gE. The gN homologs encoded by PRV and BHV-1 inhibit transporter-associated antigen processing (TAP)–mediated transport of cytosolic peptides into ER, which consequently blocks the assembly of peptide-containing ternary major histocompatibility complex (MHC)-I complexes in vitro in virus-infected cells. Furthermore, the BHV-1 gN targets the TAP complex for proteosomal degradation. The TAP complex consists of a TAP1/TAP2 heterodimer, both of which are members of the ATP-binding cassette transporter superfamily. Peptide transport by TAP is a critical step in MHC class I antigen presentation. In the absence of a functional TAP transporter, most MHC class I molecules are not loaded with peptides. However, they are retained within the ER and ultimately directed for degradation by the proteasome. Structurally, gM and TAPs are similar because they contain multiple membrane-spanning segments. A gN mutant that lacks the cytoplasmic tail can still bind to the TAP complex and block peptide transport, but this mutant gN protein does not degrade TAP. Recent results in our laboratory (Chowdhury, unpublished data) and others (Wiertz and colleagues) suggest that sequences within the gN luminal domain bind to TAP and block its peptide transport function. The ability of gN to inhibit TAP is hypothesized to prevent virus-infected cells from being killed by CD8+ T cells. Therefore, virulent field strains and modified live vaccine (MLV) viruses containing the intact gN will transiently immune suppress infected calves.

The bICP0 Protein Activates Viral Gene Expression and Inhibits the IFN Signaling Pathway

The bICP0 protein is the major transcriptional regulatory protein because it activates expression of all viral promoters, and bICP0 mRNA is constitutively expressed during productive infection. bICP0 contains a well-conserved C$\text{\textit{HC}}$4 zinc RING finger near its amino-terminus. Disruption of the bICP0 zinc RING finger prevents transactivation of a simple viral promoter, and impairs the ability of bICP0 to simulate plaque formation. The bICP0 protein also contains 2 transcriptional activation domains and a nuclear localization signal that is important for efficient transcriptional activation. Unlike most transcription factors, bICP0 does not seem to directly bind to specific DNA sequences. However, bICP0 associates with chromatin remodeling enzymes, suggesting that these interactions are necessary for activating viral transcription.

In the absence of other viral genes, bICP0 inhibits IFN signaling by directly or indirectly reducing IFN regulatory factor 3 (IRF3) protein levels in human or bovine cells. In addition, bICP0 inhibits the ability of IRF7 to activate IFN-β promoter activity, but does not reduce IRF7 protein levels. The C$\text{\textit{HC}}$4 zinc RING finger of bICP0 is an E3 ubiquitin ligase, suggesting that bICP0 degrades IRF3. IRF3 activation is an immediate early (IE) regulator of the IFN response (see Fig. 1), indicating that the ability of bICP0 to induce IRF3 degradation is important. Because IRF7 is more important to inhibiting viral infection when IRF3 versus IRF7 knockout mice are compared, additional studies were performed to understand how
bICP0 inhibits the ability of IRF7 to transactivate the IFN-β promoter. bICP0 interacts with IRF7 or a complex containing IRF7, 51 and this interaction correlates with the ability of bICP0 to inhibit the ability of IRF7 to transactivate IFN-β promoter activity (see Fig. 2B). The ability of bICP0 to interact with a chromatin remodeling enzyme (p300- or p300-containing complexes) 43 may also interfere with IFN-β promoter activity because p300 is crucial for stimulating IFN-β promoter activity.

**The Envelope Glycoprotein gG Interacts with Chemokines**

BHV-1, BHV-5, and equine herpesvirus 1 encode glycoprotein G (gG) (see Fig. 1 for location of gG), which is secreted from infected cells and can bind to a broad range of chemokines. 52 Chemokines are small proteins (8–10 kDa) that function as cytokines, and thus regulate trafficking and effector functions of leukocytes. 53 As such, chemokines are important regulators of inflammation and immune surveillance, and they have potent antiviral functions. Functionally, chemokines are divided into 2 groups: proinflammatory chemokines, which are inducible, and housekeeping chemokines, which are constitutively expressed. Activation of chemokine functions is dependent on selective recognition and activation of chemokine receptors.
Interactions between gG and chemokines block chemokine activity by preventing their interactions with specific receptors. Consequently, gG disrupts chemokine gradients, which control the local environment surrounding an infected cell. A BHV-1 gG deletion mutant was reported to have reduced virulence, suggesting gG is a viral immune evasion gene. However, the exact role of gG in virulence requires additional studies because the gG mutant that was examined was not rescued, and expression of surrounding genes was not examined.

**BHV-1 Infection Triggers Cytokine Expression that Increases the Detrimental Effect of Mannheimia haemolytica Leukotoxin**

As noted earlier, respiratory infection with BHV-1 predisposes cattle to secondary bacterial pneumonia due to infections or colonization by bacteria including *Mannheimia haemolytica*. *M haemolytica* leukotoxin is the major factor that contributes to lung injury in bovine pneumatic pasteurellosis. A recent study concluded that BHV-1 infection of bovine bronchial epithelial cells triggers cytokine expression, which subsequently promotes neutrophil recruitment to the lung. As a result, the detrimental effects of *M haemolytica* leukotoxin are amplified.

** IMMUNE RESPONSE TO BHV-1 FOLLOWING ACUTE INFECTION **

Although BHV-1 can cause transient immunosuppression in cattle, a potent immune response eventually occurs during acute infection, which prevents systemic infection. With respect to BRDC, this implies that immunosuppression initiated by BHV-1 is short-lived.

The host immune response to BHV-1 infection includes innate and adaptive immune responses. Innate immune responses include the antiviral action of IFN, alternative complement pathway, and local infiltration of lymphoid cells, macrophages, neutrophils, or natural killer (NK) cells, for example. Following BHV-1 infection, IFN-α and IFN-β are detectable in nasal secretions as little as 5 hours after infection, reaches a maximum level at 72 to 96 hours after infection, and can persist for up to 8 days after infection. Soon after infection, IFN-α and IFN-β promote leukocyte migration, activate macrophages, and increase NK cell activity. Activation of macrophages and increasing NK cell activity stimulates cytolytic activities against virus-infected cells. NK cells are a diverse population of nonadherent effector cells that lack T and B cell markers. These cells require a long incubation period with the target cell for optimum lysis. In addition, NK-like cytotoxicity is also associated with a population of CD3+ CD45+, Fc receptor-positive lymphocytes, which may represent a subset of γδ T cells.

Adaptive or humoral immune responses lead to production of neutralizing antibodies that bind virus particles and inhibit productive infection. Envelope glycoproteins gB, gC, gD, and gH are the most potent inducers of virus-neutralizing antibodies. Non-neutralizing antibody may also mediate the destruction of enveloped virus or cells expressing viral proteins on the cell membranes, and this process is referred to as antibody-mediated cell cytotoxicity. Neutralizing and non-neutralizing antibodies produced against envelope proteins can inhibit virus infection by several distinct mechanisms: (1) membrane attack complex lysis of virus envelope and virus-infected cells mediated by antibody and complement, and (2) antibody-mediated cell cytotoxicity in which IgG interacts with Fc receptor–positive cells (macrophages), or (3) binding of C3b to IgM mediates binding to C3b receptor–positive cells (lymphocytes, macrophages). In all cases, virus-infected cells are lysed. Production of virus-neutralizing and non-neutralizing antibodies (IgG) can be detected.
8 to 12 days after infection. Local/mucosal immunity depends on secreted neutralizing antibodies (IgA molecules) and systemic humoral immunity depends on IgG.

Cell-mediated immune (CMI) responses play an important role in killing virus-infected cells that express viral antigens on the cell surface. A CD8+ cytotoxic T-lymphocyte (CTL) response is an important defense against BHV-1 because cell-to-cell spread in upper respiratory epithelium occurs before hematogenous spread. Cytotoxic and proliferative T-lymphocyte responses are detected in circulating blood approximately 8 days after infection. CTL responses are induced by gB and gD DNA vaccines in mice. gC and gD have been identified as targets for CTL responses in cattle, and gB DNA vaccines elicit a CTL response in cattle. Other structural and nonstructural viral proteins may also play a role in CMI response because only a limited number of BHV-1 proteins have been evaluated for CTL activity. In addition to destruction of infected cells, T lymphocytes release several lymphokines that modulate specific and nonspecific immune responses. For example, IFN-γ and other factors that further activate macrophages are produced. BHV-1 proteins (gB-, gC-, gD-, and VP8) are recognized by CD4+ T helper cells from immune cattle. Cells expressing gB, gC, or gD on their membranes have also been identified as targets for CD4+ T cells. CD4+ T cells are important for the development of antibody response and for developing effective CD8+ T-cell memory. Thus, CD4+ T cells, CD8+ T cells, and antibodies are required for long-term protection.

THE BHV-1 LATENCY-REACTIVATION CYCLE OF BHV-1

Acute Infection Leads to High Levels of Virus Production

BRDC is not necessarily associated with acute BHV-1 infection. Although this clearly indicates that additional cofactors exist for developing BRDC, the ability of BHV-1 to reactivate from latency can also induce BRDC. Thus, the latency-reactivation cycle complicates the control of BRDC.

Acute BHV-1 infection is initiated on mucosal surfaces and results in high levels of programmed cell death. Infection of permissive cells with BHV-1 also leads to rapid cell death, in part due to apoptosis. Viral gene expression is temporally regulated in 3 distinct phases: IE, early (E), or late (L). IE gene expression is stimulated by a virion component, α-TIF. Two IE transcription units exist: IE transcription unit 1 (IEtu1) and IEtu2 (see Fig. 1A). IEtu1 encodes 2 proteins, bICP0 and bICP4, which are activators of transcription. IEtu2 encodes a protein called bICP22. In general, IE proteins activate E gene expression, and then viral DNA replication occurs. L gene expression is also activated by bICP0, culminating in virion assembly and release. As discussed earlier, bICP0 is important for productive infection because it transcriptionally activates all viral promoters, and is expressed at high levels throughout infection. Acute infection leads to high levels of virus production and secretion in ocular, oral, or nasal cavities. If acute infection is initiated in the genital tract, virus shedding can be readily detected in genital tissues. Regardless of the site of infection, virus shedding lasts for 7 to 10 days after infection.

Summary of the Latency-Reactivation Cycle in Cattle

Viral particles enter the peripheral nervous system via cell-to-cell spread. If infection is initiated via the oral cavity, nasal cavity, or ocular orifice, the primary site for latency is sensory neurons within trigeminal ganglia (TG). High levels of viral gene expression or infectious virus are detected in TG from 1 to 6 days after infection (Fig. 3 gives a summary of the latency-reactivation cycle of BHV-1). Viral gene expression and detection of infectious virus is subsequently extinguished, but viral genomes are
detected in TG (establishment of latency). In contrast to productive infection in cultured cells, a significant number of infected neurons survive, and these neurons harbor latent genomes. A hallmark of latency is the abundant level of transcription that occurs from the latency-related (LR) gene. It seems that the LR transcript is the first viral transcript expressed in infected neurons, suggesting that LR gene products play a pivotal role in programming the outcome of virus infection in sensory neurons. Support for this prediction comes from the finding that LR gene products promote establishment of latency by inhibiting apoptosis and viral gene expression. During maintenance of latency, infectious virus is not detected using standard virological methods, but the LR gene is abundantly expressed.

Increased corticosteroid levels (stress) or immune suppression can initiate reactivation from latency. The stress associated with moving cattle from one location to another is an obvious stimulus that can trigger reactivation from latency and BRDC. During reactivation from latency, 3 significant events occur: (1) productive viral gene expression is readily detected in sensory neurons, (2) LR gene expression decreases, and (3) infectious virus is secreted from ocular and nasal cavities. Administration of dexamethasone to calves or rabbits latently infected with BHV-1 reproducibly leads to activation of viral gene expression and reactivation from latency. Although many latently infected neurons do not produce infectious virus, higher numbers of neurons express lytic viral genes, indicating that virus-producing neurons are rare. For a summary of the steps involved in the latency-reactivation cycle, see Fig. 3.

**Non-Neural Sites of Latency Persistence in Cattle**

Although establishment of latency in ganglionic neurons is the main site of BHV-1 latency, viral DNA is present in tonsils, peripheral blood cells, lymph nodes, and spleen of latently infected calves, even when infectious virus is not detected. PRV, equine herpesvirus type 4, and canine herpesvirus type 1 DNA are also detected in lymphoid tissue during latency. It is not yet known which non-neural cell types are latently infected with BHV-1, and whether viral genes are expressed in these latently infected cells. In contrast to latency in sensory neurons, LR-RNA is not abundantly expressed in latently infected lymphoid tissue. Infectious virus can be detected when germinal centers from tonsil of latently infected calves are explanted, adding support to the concept that BHV-1 establishes a latent or persistent infection in cells of lymphoid origin.
Proteins Encoded by the LR Gene are Necessary for the Latency-Reactivation Cycle

As discussed earlier, LR-RNA is abundantly transcribed in latently infected neurons, and is antisense relative to the bICP0 gene. The LR gene has 2 ORFs (ORF1 and ORF2). A peptide antibody directed against ORF2 recognizes a protein encoded by the LR gene. LR gene products inhibit cell proliferation, bICP0 RNA expression, and apoptosis. LR protein expression (ORF2) is necessary for inhibiting apoptosis, but not cell growth or bICP0 expression.

A mutant BHV-1 strain with 3 stop codons at the N terminus of ORF2 does not express ORF2 following infection of bovine cells. Calves infected with the LR mutant virus exhibit diminished clinical signs and reduced shedding of infectious virus from the eye, TG, or tonsil. The LR mutant virus does not reactivate from latency following treatment with dexamethasone, whereas all calves latently infected with wild-type virus or the LR rescued virus shed infectious virus following dexamethasone treatment. Thus, expression of wild-type LR gene products is necessary for the latency-reactivation cycle in calves. The authors believe that LR gene products, including LR proteins, directly regulate the establishment and maintenance of latency. It seems unlikely that LR gene products directly stimulate reactivation from latency because dexamethasone reduces LR-RNA levels. LR gene products are predicted to promote neuronal survival following infection, thus increasing the number of neurons that can support reactivation for latency.

BHV-1 VACCINES

Many commercially available vaccines directed against BHV-1 can cause disease in small calves, partly because these vaccines are immunosuppressive. Consequently, certain vaccines have the potential to induce BRDC in small calves. The current status of BHV-1 vaccines and how they can be improved are discussed in the following paragraphs.

Commercially Available Vaccines

Commercially available vaccines directed against BHV-1 consist of an MLV or killed whole virus (KV). In many cases MLVs are attenuated by serial passage in tissue culture. KV vaccines are usually produced by chemical treatments, such as formaldehyde, β-propiolactone, or binary ethylenimine. MLVs generally induce humoral and cellular immune response because virus replication in infected cells leads to presentation of viral antigen on MHC class I and II molecules. Safety is a concern for MLVs because these strains can establish latency and, on reactivation from latency, can be transmitted to pregnant cows, which can lead to abortion. MLVs can also be pathogenic in small calves because their immune systems are not fully developed, and most MLVs can be immunosuppressive. KV vaccines are usually safe but they are not as efficacious because they usually produce only humoral immunity but no cellular immune responses. In addition, KV vaccines always require more than 1 injection to achieve acceptable neutralizing antibody levels. In the case of formaldehyde-inactivated KV, antigens may also be denatured, which may affect the immunogenicity of vaccine preparations. KV also requires suitable adjuvant formulations, and some adjuvants may induce injection-site reactions.

Commercially available BHV-1 vaccines are primarily evaluated on induction of neutralizing antibodies and the duration, as well as the level of virus shedding following challenge of vaccinated animals. As discussed earlier, the CMI response against the virus is important to prevent cell-to-cell spread of the virus. Therefore, a direct measure of CMI directed against a vaccine virus is important. Traditionally, IFN-γ
levels in vaccinated calves have been an indicator of cellular immune responses. However, a recent study found that there is an increase in CD25 by CD4+T, CD8+, and γδT lymphocytes from a BHV-1 MLV-vaccinated group. Therefore, increased expression of CD25, in addition to IFN-γ production by T cells after vaccination, seems to be a useful cellular immunity marker.

Development of Genetically Engineered Gene-Deleted BHV-1 Vaccines

During the last 10 to 15 years, the usefulness of genetically engineered gene-deleted vaccines has become increasingly apparent because they can be attenuated and serologically distinguished from wild-type field strains. Numerous viral mutants (gC-, gE-, gG-, and Us9-deleted; thymidine kinase [TK]–deleted; and LR gene mutant virus) have been constructed and their in vivo pathogenic properties, reactivation properties, and immunogenicity analyzed. Based on recent studies, gE- and Us9-deleted viruses were safe in calves because they do not reactivate from latency and they are highly attenuated (see Fig. 1 for location of gE and Us9 genes). Studies with gC, gG, or TK-deleted viruses showed that they reactivate from latency or they retain some degree of virulence. Studies with an LR mutant virus showed that the virus does not reactivate from latency and has reduced pathogenicity in calves. Therefore, considering the virulence and reactivation properties of gene-deleted vaccine strains, gE, Us9-deleted, or the LR mutant virus have the potential to be safer vaccine candidates. Comparative vaccine efficacy studies showed that, relative to gC- and gG-deleted viruses, gE-deleted virus is less efficacious. However, the gE-deleted vaccine has been used successfully to eradicate IBR from several European countries. Although the gE-deleted marker vaccine is not as efficacious as others noted earlier, it will be used until a better genetically engineered vaccine is developed.

Recent efforts to improve the IBR marker vaccine are directed to deleting viral gene sequences that are immunosuppressive. As noted earlier, gN inhibits TAP and down-regulates MHC-I antigen presentation. Therefore, a vaccine virus lacking the gN TAP-binding domain may stimulate better cellular immune responses. Our recent studies show that a gE cytoplasmic tail truncated virus is attenuated in calves infected with the virus similarly to the entire gE ORF-deleted virus. Notably, like the gE-deleted virus, the gE cytoplasmic tail truncated virus does not reactivate from latency. Because the cytoplasmic tail–specific amino acid sequences generate antibodies that immunoprecipitate gE, antibodies specific to the cytoplasmic tail sequence may serve as serologic markers to distinguish vaccinated versus infected calves. Furthermore, the Us9 gene is located immediately downstream of the gE cytoplasmic tail (see Fig. 1), so a recombinant BHV-1 can be constructed in which the gE cytoplasmic tail and the Us9 coding regions are deleted. A vaccine virus that lacks US9, the cytoplasmic tail of gE, and gN TAP-binding domain may be a superior vaccine candidate because (1) it should stimulate BHV-1–specific CMI responses more efficiently, (2) it will incorporate a serologic marker, and (3) it should not be pathogenic. Concerns have been raised about recombination occurring between 2 vaccine strains, which could lead to the presence of virulent viral strains in vaccinated herds. Although it is clear that recombination can occur, the process requires that both viruses must replicate at the same time in the same cell of an infected calf.

Problems Associated with Current Modified Live BHV-1 Vaccines and BHV-1 Eradication Effort

There has recently been an apparent increase in IBR outbreaks in vaccinated feedlot cattle (commonly referred to as vaccine outbreaks). Many of these vaccine
outbreaks occur in feedlots that have used several different BHV-1 MLVs without serologic markers, suggesting that they are not vaccine specific. Because the vaccines used in the feedlots did not have any serologic marker, it is difficult to determine by serology whether an animal is infected with an MLV or field strain. It is also possible that recombination between MLV strains could lead to a virus with increased pathogenic potential. Thus, determining the source of origin from a particular vaccine break is not possible, nor is it possible to test whether these outbreaks were due to changes in the vaccine strain. The emergence of a new IBR strain that is not covered by existing MLVs would also lead to clinical outbreaks in herds in which vaccination has occurred. A study investigating several isolates from such outbreaks determined that at least 1 such isolate had mutations within sequences comprising the gD-specific neutralizing epitope.127

In several European countries (Austria, Denmark, Finland, Norway, Sweden, Switzerland), IBR has presumably been eradicated by the use of gE marker vaccines. In these countries, until recently, cattle were no longer vaccinated against IBR (see later discussion). In several other EU countries, including Germany, The Netherlands, and the United Kingdom, the same strategy has been in use to eradicate the disease. However, IBR-free status for these countries has not yet been possible. Recently, new IBR cases have been recorded in several supposedly IBR-free countries. One such outbreak in Switzerland was tracked back to the importation of latently infected animals.128 Previously, importation of BHV-1–positive semen was shown to be the cause of IBR outbreaks.128

Because the severity of IBR disease in immunologically naïve animals is more pronounced, outbreak of a virulent IBR could have severe consequences. Therefore, IBR-free countries are endangered as long as IBR eradication is not a common goal within countries that are involved in cattle trade and production of semen for artificial insemination. Currently, in these countries, vaccination with gE-deleted marker vaccine is again being allowed. Identification and characterization of the new isolates by a clustering system using DNA restriction profiles with HpaI, HindIII, SfiI, and PstI are being pursued. Strict import regulation, regular immunologic surveillance, tracing the source of an IBR outbreak, and a continued vaccine program will be necessary to control the disease.

SUMMARY

BRDC is the primary disease of cattle in the United States. Although it is recognized as being a multifactorial disorder, BHV-1 is known to play an important role in BRDC. The ability of BHV-1 to inhibit the immune system plays a role in allowing opportunistic bacterial infections to induce clinical disease. Furthermore, the tissue damage caused by BHV-1 acute infection also makes it possible for bacterial infections to spread throughout the upper respiratory tract. The ability of BHV-1 to establish a latent infection in sensory neurons confounds treatment of BHV-1 related diseases. Development of vaccine strains that are not immunosuppressive and do not reactivate from latency would help to eliminate the clinical disorders caused directly and indirectly by BHV-1.

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