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infection of cattle

Laurel J. Gershwin*

Department of Pathology, Microbiology, & Immunology, University of California, Davis, CA 95616, United States



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Department of Pathology, Microbiology, & Immunology, University of California, Davis, CA 95616, United States

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ABSTRACT

Bovine respiratory syncytial virus (BRSV) is a respiratory pathogen of cattle that causes severe disease in calves alone and as one of several viruses and bacteria that cause bovine respiratory disease complex. Like human RSV this virus modulates the immune response to avoid stimulation of a vibrant CD8+ T cytotoxic cell response and instead promotes a Th2 response. The Th2 skew sometimes results in the production of IgE antibodies and depresses production of the Th1 cytokine interferon γ . Innate immune cells have a pivotal role in guiding the adaptive response to BRSV, with selective secretion of cytokines by pulmonary dendritic cells. Here we review some of the pertinent observations on immune responses to BRSV infection and vaccination and illustrate how experimental infection models have been used to elucidate the immunopathogenesis of BRSV infection. Recent experiments using intranasal vaccination and/or immune modulation with DNA based adjuvants show promise for effective vaccination by the stimulation of Th1 T cell responses.

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1. Introduction

Bovine respiratory syncytial virus is a single negative stranded RNA virus in the genus pneumovirus and family paramyxoviridae. It causes respiratory disease in cattle, as a single disease agent and as a component of the bovine respiratory disease complex (BRDC). BRSV is particularly virulent for young calves and less so for more mature cattle [1]. This situation parallels that of respiratory syncytial virus, a closely related human pathogen. Of the 11 viral proteins, the envelope of the virus contains three transmembrane glycoproteins: the largest glycoprotein (G) protein, which is involved in cell attachment; the fusion (F) protein, and the small hydrophobic (SH) protein. The fusion protein causes fusion of cell membranes with resultant formation of syncytia and by so doing it facilitates movement of the virus between cells. The viral proteins that have been most associated with protective immunity are the major surface glycoprotein, or G protein; the fusion

protein, and the nucleoprotein [2]. Infected cattle make antibody responses to all three of these proteins, as well as some of the minor viral proteins. BRSV is a fairly genetically stable virus. However, some changes in the sequence have evolved over time in the N, G, and F proteins possibly due to pressure from vaccination [3]. Nonetheless repeated infection not only of a population but also of an individual is common in both human and bovine RSV infection. Neither RSV nor BRSV infection appear to evoke a strong immunological memory response [4].

The disease caused by BRSV begins with a fever, cough, and often a mucoid nasal discharge. The fever increases sometimes to as high as 106 °F. This is accompanied by depression, increased respiratory rate, and anorexia. Auscultation of the lungs in the most severe cases will show the presence of wheeze. Open mouth breathing with extended head and neck is characteristic of the most severe form of the disease. In less severe cases a several days of fever and cough may be the only clinical signs observed. This breadth of disease severity is also seen in human RSV infection where the disease can vary from a mild cold to a severe interstitial pneumonia requiring hospitalization and intensive care. The role of maternal antibody in protection from

* Tel.: +1 530 752 6643; fax: +1 530 752 3349.
E-mail address: ljgershwin@ucdavis.edu

BRSV infection has been studied with varied results. It is generally true that the presence of maternal antibody to BRSV is not fully protective, while high passively acquired titers dampen the disease process in human RSV infected infants treated with high titer RSV specific monoclonal antisera, palivizumab, a licensed product for reduction of disease severity in high-risk infants [5].

Infection with BRSV initiates an IgG response as well as IgA in respiratory secretions; the cell mediated response is has been less well defined, but those studies in which it was evaluated have not shown it to be vigorous and long lasting. The most interesting aspect of the immune response to BRSV is that in some individuals IgE antibodies are made against viral proteins. Indeed, when anti-BRSV IgE is present the disease is greater in severity. The production of IgE against viral proteins was also shown in human children with severe disease and wheezing during RSV infection [6]. We showed a correlation between BRSV-specific IgE and increased clinical sign scores during experimental infection [7].

2. Innate immunity and BRSV infection

Recent interest in the role of dendritic cells as sentinels that interact with pathogen associated molecular motifs (PAMPS) has focused on early production of proinflammatory cytokines and chemokines that direct subsequent adaptive immune responses. The interaction between BRSV and dendritic cells within the respiratory mucosa is an important step in modulating the immune response towards either a T helper 1 (TH1) or T helper 2 (TH2) type response. In one study bovine dendritic cells were stimulated *in vitro* with RSV and with LPS. Using RT-PCR transcripts for RANTES, MIP-1 α , MIP2 α , MIP3 α , MCP-2, and the cytokine receptor CCR3 were detected [8]. The fusion protein (F protein) of RSV and BRSV interacts with toll-like receptor 4 (TLR4) and is thought to help initiate the innate immune response. The involvement of other cell surface molecules, MD2 and CD14, in stimulating the TLR-4-dependent NF-kappa B activation was suggested [9]. This mechanism is similar to cellular activation by lipopolysaccharide (LPS), which binds to CD14/TLR4 complex to activate macrophages and initiate cytokine production. This similar early signaling is important in activation of the innate immune response to BRSV. Microarray analysis of gene expression in BRSV infected bovine respiratory/turbinate epithelial cells demonstrated up-regulation of the proinflammatory cytokines IL-6 and IL-8 (Gershwin et al. unpublished data). These data are in agreement with a study on human nasal epithelial cells from RSV infected patients that showed expression of IL-6, IL-8 and RANTES [10]. The pro-inflammatory cytokines, IL-1, IL-6, and TNF α are responsible for “sickness behavior” [11]. These cytokines act on the brain to modify behavior, creating anorexia, fever, and a desire to sleep – all among the earliest signs of BRSV infection in experimentally infected calves. These cytokines are important in recruiting additional inflammatory cells to the area of viral entry. IL-8 is believed to play a role in the pathogenesis of bronchiolitis, which is typically seen in BRSV infection. It is chemotactic for neutrophils.

IL-6 is an important mediator of fever, as it is able to cross the blood brain barrier and change the temperature “set-point”. Thus early interaction of BRSV with cells of the innate immune system initiate the disease process and begin to trigger an adaptive immune response. The critical step in Th2 bias of the BRSV/RSV immune response is initiated at the level of the dendritic cell, which fails to produce a strong IL-12 response to initiate Th1 cell proliferation. Studies with human RSV have shown that the interaction with the virus and the pulmonary dendritic cells reduce the capacity for production of key cytokines for an effective anti-viral T cell response [4].

3. The immune response to experimental and field BRSV exposure

Many studies were performed using experimental infection of calves with BRSV. The studies differ in that some use conventional calves, others use gnotobiotic calves, and some use colostrum deprived calves. The presence of maternal antibodies has not been found to be protective, although there is some indication that they can suppress the immune response to infection. Using caesarian-section derived 1 month old calves; Kimman et al. experimentally infected calves with BRSV and evaluated isotype-specific immune responses. Colostrum-deprived calves showed BRSV-specific IgM and IgA in serum, lung lavage fluid, and nasal secretions as early as 8 days after infection. IgG₁ appeared between days 13 and 17 post-infection, while serum IgG₂ failed to appear until 1–3 months after infection [12]. In lung lavage fluid both IgM and IgA were detected between days 8–18, and 10–13, respectively. A similar study reported by this group in which colostrum fed calves were used showed that calves with high maternal antibody titers failed to produce more than a trace of IgM, IgA, and IgG when infected. However, upon reinfection 3 months later strong IgA responses were observed as well as IgG₁ and IgG₂ [12]. The Kimman study measured antibody titers using ELISA methodology.

In another study Knott et al. used specific pathogen free calves to study immune responses to a BRSV isolate that had never been passaged on cell culture. Using both ELISA and virus neutralization (SVN) assays they found that antibodies were detected by both assays after day 14. Titers on the day of infection were <5 for BRSV-specific IgG₁ ELISA and 0 for the SVN assay. Titers on day 21 varied from 66 to 474 (ELISA) and from 20 to 160 (SVN) [13].

4. Immune modulation by BRSV

Using an experimental infection protocol developed in our laboratory we have studied a variety of immunological parameters of host response to BRSV infection. The model produces moderate to severe disease in Holstein calves from 6 to 16 weeks of age; and mild to moderate disease in 6–8 month old Angus-cross cattle. Briefly, low passage clinical isolate of BRSV (CA-1) is aerosolized to cattle using a facemask with a nebulizer and DeVilbiss air compressor. Clinical signs are evaluated and scored daily using modifications of the system previously developed by Collie [14]. Infected calves typically develop a fever on day

4–5 after infection with cough beginning as early as day 3 and lasting for at least 10 days. Abnormal lung sounds are auscultated from day 5 through day 10 and consist of harshness and sometimes wheezes and rales. Virus is shed from the nasal passages typically from day 4 through day 10, and sometimes is detectable as early as day 1, which is likely residual from infection. In those cases that are severe open mouth breathing and dyspnea the PaO₂ is decreased significantly from baseline and lung compliance is significantly increased [15].

Studies with human RSV have shown that the basis for the inefficient immune response triggered by the virus is a result of induction of a Th2-biased response specific for some RSV antigens. Further observations that RSV infection favors induction of an allergic type response and a decreased Interferon γ [4].

Using our experimental infection model we showed that during a ten day course of infection, there was an early peak in IL-4 production (at day 4). These observations were performed on efferent lymphocytes from the lung of experimentally infected calves. The model was further expanded to allow the evaluation of lymphocytes and lymph fluid draining the lung by surgical cannulation of the caudal mediastinal lymph node [16]. Calves were infected with BRSV after cannulation and daily lymph was collected for 10 days. Earlier we had shown that there was a correlation between serum BRSV specific IgE in experimentally infected calves and severity of disease [17,18]. Twelve 6–8 week old Holstein beef calves were infected 5 days after lymph cannulation surgery. Six additional calves received mock infection and served as controls. Lymph fluid was evaluated for BRSV specific IgE by ELISA [16] and RNA was prepared from the lymph pellets. RT-PCR was performed and RNA from days –1 through 9 to evaluate RNA expression for IL-2, IL-4, and Interferon γ . Expression of IL-4 was enhanced in the BRSV infected calves. Interferon γ was also present in these calves and the majority of them show production during days 5–7 of infection and also during days 8 through 10. Control calves and infected calves showed consistent expression of IL-2 throughout the 10 days of infection [16]. These studies were semi-quantitative, but the pattern of cytokine production favored a Th2 type response and correlated with the induction of IgE antibodies.

The association between BRSV infection and allergic sensitization was further examined using the same infection and cannulation model. Lung lymphatic cannulated calves were infected with BRSV ($n=7$) or mock infected ($n=6$) on day 0. For the next 6 days each calf received an aerosol exposure to ovalbumen (a prototype allergen). Calves were evaluated for signs of disease, Ovalbumen specific IgE levels and T cell subsets in lung lymph, and cytokine expression was quantitatively measured by RT-PCR. There was a significantly greater IgE response to the ovalbumen in BRSV infected calves and on days 3 through 5 IL-4 and IL-13 gene expressions in BRSV infected calves were increased compared with mock infected controls [7].

5. BRSV and T cell mediated immunity

Removal of viral infected cells by cytotoxic T lymphocytes is a major mechanism limiting infection by a virus.

Unlike many viruses that elicit a strong CD8 cytotoxic T cell response, which results from dendritic cells secreting IL-12 to cause Th1 polarization and the production of interferon γ , BRSV infection elicits a weak and transient cytotoxic T cell response [19,20]. Studies with human RSV in rodent models have shown that a strong cytotoxic T cell response is blocked by the production of Th2 cytokines [4]. We and others have found this to be true for BRSV. In a study that involved evaluation of the cytotoxic T cell response after BRSV vaccination and challenge, Woolums et al. found that in both the unvaccinated BRSV infected control group and the formalin-inactivated vaccinated group the cytotoxic T cell response was minimal. Using a chromium release assay with autologous fibroblasts as BRSV-infected targets, the percent lysis by CTL's peaked on days 7–10 post infection and ranged from 11 to 25% [19].

6. Immune response to BRSV vaccination

The challenge of vaccination for BRSV is that a vaccine must not mimic the immune response induced by infection; it must be different and better. The realization that a killed adjuvanted RSV/BRSV vaccine would not be suitable began with the catastrophic results of vaccination of children with formalin-inactivated RSV vaccine in the 1960s, which resulted in vaccine enhanced disease [21]. Our group re-created the vaccine enhanced disease with formalin-inactivated BRSV vaccine in cattle [22]. We further showed that the production of Interferon γ was impaired in vaccinated/infected cattle compared with mock vaccinated/infected cattle and that the CTL cell mediated response to infection was minimal [19].

Unlike human RSV for which there is still no approved vaccine, there are multiple BRSV vaccines on the market, most as part of multi-antigen preparations. There have been several reports of vaccine exacerbated disease occurring in cattle as a result of natural infection of vaccinated cattle. Kimman et al. reported a severe outbreak among cattle vaccinated with a modified live vaccine in the Netherlands. They concluded that BRSV was present in the herd at the time of vaccination because there were detectable BRSV specific IgM titers. However the vaccinated cattle were more severely affected than unvaccinated cattle [23]. Another report of field exposure disease exacerbation documented severe fatal BRSV in cattle vaccinated with a killed BRSV vaccine in Belgium [24]. This vaccine was killed with β -propiolactone rather than formalin but was incorporated into an adjuvant that contained aluminum hydroxide. In our experimental reproduction of the exacerbation, as well as reports of others the reported lung lesions were reminiscent of those seen in the children that had vaccine exacerbated disease, including eosinophilia in some cases, severe proliferative alveolitis, bronchiolitis obliterans, and areas of emphysema.

The kinetics of anti-BRSV CD8+ T cell responses in formalin inactivated BRSV vaccinated/infected calves were compared to those in live attenuated BRSV vaccinated/infected calves. The live attenuated BRSV vaccine primed a T cell memory response [25].

The variety of BRSV vaccines available include killed and modified live products and it is generally thought that

these vaccines reduce the severity of bovine respiratory disease, particularly when administered as part of a multi-valent vaccine containing other bovine respiratory disease pathogens. Although the approved method for vaccination is by the parenteral route, Ellis et al. vaccinated cattle by the intranasal route with a single component modified live virus (MLV) BRSV vaccine, with a multiple antigen BRSV MLV vaccine and with a multiple antigen vaccine that lacked BRSV. They found that the intranasal administration of the MLV BRSV provided clinical protection after experimental challenge in calves vaccinated at 9 weeks as well as in those vaccinated at 2 weeks of age. The authors speculated that the intrana [26].

Formalin-inactivated BRSV vaccines and other commercially prepared killed BRSV vaccines are considered to be risky for the potential stimulation of a non-protective Th2 response and also an adverse response to infection. As stated above the immunomodulatory nature of BRSV favors this non-protective response. In an effort to alter the immune response to BRSV Oumouna et al. used a formulation of CpG oligonucleotides (odn) administered with formalin activated BRSV vaccine to mice. The control mice that received the FI-BRSV vaccine without the CpG odn produced IgG1, IgE, and IL-4 – hallmarks of the Th2 response. Those mice receiving the same vaccine with the CpG containing made odn mice show more polarized immune responses than cattle, these results suggest that such immune modulation could be a useful adjunct to BRSV vaccination protocols [27].

The idea of using a DNA vaccine coding for the nucleoprotein of BRSV as an immunogen for induction of cell mediated immunity was tested in a preliminary experiment in our laboratory. A plasmid coding either the N protein or the F protein was used to immunize 6 week calves with low BRSV specific maternal antibody titers. Control calves received plasmid containing green fluorescent protein. Experimental infection of all three groups demonstrated significantly lower clinical scores in the N protein group when compared to both the F protein and the N protein animals. Lymphocyte stimulation of peripheral blood mononuclear cells showed a significantly greater T cell response to viral antigen in the N protein vaccinated group [28]. Subsequently Letellier et al. used a DNA prime and protein boost with BRSV nucleoprotein in a vaccine trial. They found that this protocol protected from viral shedding and lung lesions. The N protein is a recognized targeted for CD8+ cytotoxic T cells [29]. Using the same rationale, another group expressed the human RSV N protein and used it as a subunit vaccine incorporated into nanoparticles to immunize calves. Their results showed that there was cross-protection and that the immunization decreased viral shedding and lung pathology after experimental infection and that there was no vaccine induced exacerbation of disease [30].

7. Summary

Our knowledge of the complex relationship between BRSV and its bovine host has increased greatly over the past decades. Observations made in cattle have been mirrored in the human-rodent models, demonstrating a strong

similarity in host–viral interactions of these species. We now appreciate that it is not the viral infection, but rather the host response to the virus that causes the most severe pathology and clinical disease. Innate immune responses have been characterized by in vitro studies that demonstrate the importance of the initial cytokine response in polarization of the acquired immune response to the virus. Unlike most viruses that generate a vigorous cytotoxic T cell response, BRSV attempts to subvert the host defense by initiation of a Th2 type response that is more “virus friendly” but which can stimulate unwanted inflammation in the infected lung. New modalities for vaccination and immunomodulation are now being applied to this fascinating host–pathogen relationship.

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