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Immunology of bovine respiratory syncytial virus in calves☆

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ABSTRACT

Bovine respiratory syncytial virus (BRSV) is an important cause of respiratory disease in young calves. The virus is genetically and antigenically closely related to human (H)RSV, which is a major cause of respiratory disease in young infants. As a natural pathogen of calves, BRSV infection recapitulates the pathogenesis of respiratory disease in man more faithfully than semi-permissive, animal models of HRSV infection. With the increasing availability of immunological reagents, the calf can be used to dissect the pathogenesis of and mechanisms of immunity to RSV infection, to analyse the ways in which the virus proteins interact with components of the innate response, and to evaluate RSV vaccine strategies. Passively transferred, neutralising bovine monoclonal antibodies, which recognise the same epitopes in the HRSV and BRSV fusion (F) protein, can protect calves against BRSV infection, and depletion of different T cells subsets in calves has highlighted the importance of $CD8^+$ T cells in viral clearance. Calves can be used to model maternal-antibody mediated suppression of RSV vaccine efficacy, and to increase understanding of the mechanisms responsible for RSV vaccine-enhanced respiratory disease.

1. Introduction

Bovine respiratory syncytial virus (BRSV) is a major cause of respiratory disease in young calves, and is responsible for significant economic losses to the farming industry throughout the world (Valarcher and Taylor, 2007). BRSV is genetically and antigenically closely related to human (H)RSV, which is the single most important cause of lower respiratory tract disease in young infants. BRSV and HRSV are enveloped, non-segmented, negative-stranded RNA viruses belonging to the genus *Pneumovirus*, within the family *Paramyxoviridae*. Of the 11 proteins encoded by the viral genome, three transmembrane glycoproteins, the large attachment glycoprotein (G), the fusion protein (F) and the small hydrophobic protein (SH), are located in the viral envelope. The matrix protein (M) is present on the inner face of the envelope, and the nucleoprotein (N), phosphoprotein (P), RNA polymerase (L) and M2-1 constitute the nucleocapsid. The M2-2 protein is expressed at low levels in infected cells; however, it is not clear if it is incorporated into virions. The two non-structural proteins, NS1 and NS2, are present in high levels in infected cells.

Although BRSV and HRSV have a highly restricted host range, the pathogenesis and epidemiology of infection by these viruses have many features in common. In temperate climates, both viruses cause annual winter outbreaks of respiratory disease, with a peak incidence of severe disease in naïve infants and calves 1–6 months of age. The majority of calves and children become infected by 1–2 years of age (Stott and Taylor, 1985). BRSV and HRSV replicate primarily in ciliated airway epithelia cells and type II pneumocytes (Johnson et al., 2007; Viuff et al., 1996; Welliver et al., 2008), and induce a wide range of proinflammatory cytokines and chemokines (Bermejo-Martin et al., 2008; Rosenberg and Domachowske, 2012; Valarcher and Taylor, 2007), which direct the expression of cellular adhesion molecules and recruit neutrophils and lymphocytes to the lung, resulting in bronchiolitis and interstitial pneumonia. While no single animal model system can fully reflect the disease in another species, as a natural host of BRSV infection, the calf provides significant advantages over semi-permissive animal models for the study of the immunology and pathogenesis of RSV infection. Species-specific differences in host molecules involved in virus replication and differences in the way the virus interacts with components of the innate immune response may be responsible for the reduced efficiency of virus replication seen in non-native hosts and may influence the outcome of RSV infection. BRSV infection of calves therefore provides an ideal model to study the pathogenesis of and

Abbreviations: BRSV, bovine respiratory syncytial virus; HRSV, human RSV; BAL, bronchoalveolar lavage; rVV, recombinant vaccinia virus; rBHV-1, recombinant bovine herpesvirus -1; ALC, afferent lymph dendritic cells; APC, antigen-presenting cells; moDC, monocyte-derived dendritic cells; mAbs, monoclonal antibodies; MDA, maternally-derived antibodies; FI-HRSV, formalin-inactivated human respiratory syncytial virus.

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mechanisms of immunity to RSV. The large size of calves allows for frequent collection of blood and mucosal secretions from individual animals to analyse kinetic changes in the immune response, and to determine the role of cells present in low frequency in the development of immune responses. In addition, cells involved in mucosal immune responses can be analysed by taking serial biopsies from mucosal tissues by endoscopy or by surgical cannulation of lymphatic vessels that drain the oro-nasopharynx and lungs.

2. Innate immune responses to BRSV

BRSV infection induces a well-defined pathology which begins with a fever, cough, and often a mucoid nasal discharge. The fever, which can increase to as high as 40 °C, is accompanied by depression, increased respiratory rate and anorexia. Auscultation of the lungs in the most severe cases will show the presence of wheeze. Overproduction of mucus is particularly detrimental as the airways can quickly become obstructed. Whilst an appropriate innate immune response can be beneficial, uncontrolled responses lead to disease.

RSV initially encounters the respiratory epithelia and elements of the innate immune response are activated resulting in the induction of pro-inflammatory cytokines and chemokines. Increased levels of expression of IL-12, IFNγ, TNFα, IL-6, IL-18, IL-8, RANTES, MCP-1, MIP-1α, IFNα and IFNβ mRNA have been detected in pneumonic lesions from BRSV-infected calves (Sacco et al., 2012; Valarcher and Taylor, 2007). Similarly, TNF-α and IFNγ have been detected in bronchoalveolar lavage (BAL) from BRSV-infected calves with clinical signs of respiratory disease (Antonis et al., 2010). In agreement with data observed in human epithelial cells, microarray analyses of gene expression in BRSV-infected bovine turbinate epithelial cells have also shown up-regulation of pro-inflammatory cytokines (Das et al., 2005; Gershwin, 2012).

2.1. Recognition of RSV by Pattern Recognition Receptors

Studies in small animal models of RSV infection have indicated that RSV activates a number of Toll-like receptors (TLRs) on hematopoietic cells. However, since TLRs are species-specific, data obtained in mice does not necessarily relate to other species, including man and cattle. TLR2 is involved in the recognition of a wide array of microbial molecules from bacteria, mycoplasma and yeast. HRSV interacts with mouse TLR2 and TLR6 promoting neutrophil migration and dendritic cell (DC) activation within the mouse lung (Murawski et al., 2009). However, the extracellular domain of TLR2, which is responsible for ligand recognition, is significantly different in mice, man and cattle (Willcocks et al., 2013), suggesting that the effects observed in laboratory animals may not apply to man or cattle. TLR3 recognises intracellular dsRNA and although the production of dsRNA during RSV replication has not been demonstrated directly, the TLR3 pathway is activated in HRSV-infected mouse cells, resulting in chemokine upregulation (Rudd et al., 2005) and increased sensitivity to other TLR3 ligands (Groskreutz et al., 2006). Although the effect of TLR3 activation in bovine cells has not been extensively studied, BRSV induces the expression of MCP-1, MIP-1α and IL-10 mRNA in γ/δ T cells apparently in response to TLR3 activation (McGill et al., 2013).

TLR4 and its co-receptor CD14 have been shown to interact with HRSV in both human and mouse immune cells. TLR4-null mice infected with HRSV showed delayed clearance of HRSV compared to wild type mice (Kurt-Jones et al., 2000) and it was later shown that the RSV F protein activates the TLR4/CD14 complex resulting in NF-κB-mediated inflammation in both mouse and human cells (Awomoyi et al., 2007; Haeberle et al., 2002; Marr and Turvey, 2012). However, a clear role for BSRV F in stimulating NK-κB has not been defined (Lizundia et al., 2008).

TLR7 has also been proposed to mediate responses against RSV. Activation of TLR7 in HRSV-infected mouse macrophages results in increased expression of interleukin (IL)-12 and IL-23, two important T cell differentiation factors (Lukacs et al., 2010). Although ssRNA can activate bovine TLR7 *in vitro*, activation of TLR7 by BRSV has not yet been investigated (Buza et al., 2008).

In HRSV-infected infants, cytokines and chemokines such as TNFα, IL-1β, IL-6, IL-8 MIP-1α and RANTES released during RSV infection by both airway epithelial cells and lung macrophage populations (Becker et al., 1991; El-Sahly et al., 2000) have been shown to correlate with disease severity. Bovine dendritic cells (DC) and monocytes infected with BRSV showed increased levels of RANTES, MIP1α–2α–3α, MCP-2 mRNA (Werling et al., 2002b) and increased production of TNFα and IL-1β (Taylor et al., 2014), suggesting that the mechanisms involved in cytokine production by RSV infection are conserved in humans and cattle.

IL-1β, which is produced by BRSV- and HRSV-infected macrophages and epithelial cells (Bermejo-Martin et al., 2008; Fach et al., 2010; Taylor et al., 2014; Werling et al., 2002a), plays an important role in inflammation by orchestrating the pro-inflammatory response. IL-1β and IL-18, which enhance IL-12 and IFNγ production and regulate innate and acquired immune responses, are produced as cytosolic precursors which require proteolytic cleavage induced by the inflammasome for activation and secretion. HRSV activates the nucleotide-binding oligomerization domain like receptor 3 (NLR3)/ASC inflammasome. In mouse bone marrow macrophages, this is initiated by TLR-2/Myd88/NF-κB signalling, and reactive oxygen species (ROS) and K^+ ion efflux (Segovia et al., 2012). However, studies in human lung epithelial cells indicated that activation of the NLRP3/ASC inflammasome by HRSV was initiated by TLR-4 (Triantafilou et al., 2013). The mechanisms involved in induction of IL-1β in BRSV-infected cells have not yet been analysed.

2.2. Type I interferons

Type I IFNs are specialised cytokines that are released by host cells in response to pathogens. Various pattern recognition receptors (PRR) such as RIG-I receptors, TLR, NOD-like receptors, detect RSV infection and induce the production of type I IFN which in turn has antiviral effects on neighbouring uninfected cells. However, RSV is more resistant to the anti-viral effects of type I IFNs than other paramyxoviruses (Atreya and Kulkarni, 1999; Schlender et al., 2000) and NS1 and NS2 of both human and bovine RSV have been shown to suppress induction of type I IFN induction *in vitro* (Bossert et al., 2003; Schlender et al., 2000). HRSV and BRSV with single or double deletions of the NS genes (ΔNS1, ΔNS2 and ΔNS1NS2) have a reduced ability to replicate in cell culture and in animals (Bossert et al., 2003; Jin et al., 2000; Valarcher et al., 2003). NS1 and NS2 antagonize both the cellular antiviral response as well as the induction of IFN. The NS proteins appear to function independently and in combination to target a number of different factors involved in the IFN induction and response pathways (Barik, 2013). Although both HRSV and BRSV NS proteins inhibit activation of IRF3 (Bossert et al., 2003; Spann et al., 2005), the mechanisms by which these viruses inhibit IFN may differ. Thus, HRSV lacking NS1 (HRSVΔNS1) is more effective at inhibiting induction of IFN than HRSVΔNS2 (Spann et al., 2004), which is the converse of the relative contributions of the individual BRSV NS proteins to inhibition of IFN induction (Valarcher et al., 2003).