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EXPERIMENTALLY INDUCED DISEASE

Induction of Interleukin-8 and Interleukin-12 in Neonatal Ovine Lung Following Experimental Inoculation of Bovine Respiratory Syncytial Virus

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Summary

This study aimed to determine the immunohistochemical expression of interleukin (IL)-1β, tumour necrosis factor alpha (TNF)-α, interferon (IFN)-γ, IL-4, IL-6, IL-8, IL-10 and IL-12 and to measure the concentrations of these cytokines in lung tissue from lambs infected experimentally with bovine respiratory syncytial virus (BRSV). Lambs ($n = 15$) were inoculated at 2 days of age with 20 ml of viral inoculum (1.26×10^6 $TCID_{50}$ per ml) or sterile medium ($n = 15$). Rectal temperature, pulse and respiratory rates were monitored daily in control and infected lambs. Lambs were killed and subject to necropsy examination at 1, 3, 5, 7 and 15 days post inoculation (dpi). There was a temporal association between pulmonary expression of these cytokines and lung pathology in BRSV-infected lambs. The cytokines IL-4 and IL-10 were not elevated, but there was a significant increase in IL-1β, TNF-α, IFN-γ and IL-6 proteins and labelled cells, suggesting that these cytokines may play a role in the biological response to BRSV infection and contribute to the development of lung lesions. There was also a significant increase in the cytokine concentration and number of immunolabelled cells expressing IL-8 and IL-12 in infected lungs, suggesting that these cytokines might be used as therapeutic targets in the management of BRSV, in conjunction with measures to combat the causative pathogen and prophylactic methods aimed at preventing infection.

Keywords: bovine respiratory syncytial virus; cytokines; lamb; lung

Introduction

Respiratory syncytial virus (RSV), an enveloped, non-segmented, negative-strand RNA virus, is a member of the Paramyxoviridae family (Kovacs-Nolan *et al.*, 2009). RSV is the most common cause of severe lower respiratory tract infections in human infants and young calves (Scott *et al.*, 1978). The pneumovirus, bovine respiratory syncytial virus (BRSV), was first detected in 1970 (Paccaud and Jacquier, 1970; Wellemans *et al.*, 1970). BRSV is genomically, antigenically and functionally related to human RSV (HRSV), and is host adapted for ruminant infection, causing significant pulmonary lesions and clinical disease (Lehmkuhl and Cutlip, 1979; Meyerholz *et al.*, 2004).

Lambs are susceptible to infection by both ovine and bovine strains of RSV (Masot *et al.*, 1995, 1996, 2000) and can be infected experimentally with the HRSV A2 strain. Sow *et al.* (2011a,b) have reported that preterm lambs infected with HRSV develop clinical signs and lesions that parallel the course of disease observed in human infants suffering from severe RSV disease. The aim of the present study was to investigate the pathogenesis of BRSV infection in the neonatal lamb model. Sow *et al.* (2011b) described the advantages of this model over the rodent model for the study of the pathogenesis

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and immune response to HRSV infection in neonates. The choice of the neonatal lamb model for experimental infection with human and bovine strains of RSV is based on the fact that airway structure and function in neonatal lambs are similar to human infants (Plopper *et al*., 1983; Scheerlinck *et al*., 2008).

The pathogenesis of RSV disease has been studied extensively and is thought to involve a multifactorial process that includes virus-induced pathology and a concomitant exaggerated host immune response (Sow *et al*., 2011a,b). RSV-specific immunity is also believed to play a crucial role in the development of severe lower respiratory tract disease. It has been suggested that the pathogenesis of RSV infection is a combination of host proinflammatory cytokine production and the cytopathic effects of viral replication (van Schaik *et al*., 2000). Lung epithelial cells are a primary site for RSV replication, but data from research focusing on monocyte-derived dendritic cells and alveolar macrophages indicate that in-vitro replication of the virus also occurs in other cell types (Fach *et al*., 2007).

A number of authors have highlighted the role of inflammatory cytokines in the development of lung lesions associated with HRSV (Olivier *et al*., 2011; Li *et al*., 2012) and BRSV (Mclnnes *et al*., 1998; Sow *et al*., 2011a,b). Experiments in a mouse model have shown that priming with live RSV infection induces a T helper (Th) 1 response, while priming with inactivated virus induces a Th2 response when mice are challenged subsequently with live RSV (Graham *et al*., 1993). Like HRSV, BRSV modulates the immune response to avoid stimulation of a $CD8^+$ T cytotoxic cell response and instead promotes a Th2 response (Gershwin, 2012).

Werling *et al*. (2002) suggested that the level or spectrum of cytokines produced by dendritic cells, as compared with other antigen-presenting cells following exposure to live or inactivated BRSV, might play an important role in the generation of effective immune responses and modulation of the Th1/Th2 balance.

Grell *et al*. (2005a,b), in a study of age-dependent differences in the response to BRSV infection, reported a marked induction of tumour necrosis factor (TNF)-α and interferon (IFN)-γ in young calves, but not in older calves. Mclnnes *et al*. (1998) detected mRNAs for IFN-γ, interleukin (IL)-2, IL-4 and IL-10 in pulmonary and peripheral blood mononuclear cells (PBMCs) from calves with extensive pneumonic consolidation in response to BRSV infection, but only for IFN-γ in PBMCs from uninfected controls.

Fach *et al*. (2007) reported the induction of immunomodulatory IL-4 and IL-10 cytokine gene transcription in lung dendritic cells and alveolar macrophages in response to experimental RSV infection in newborn lambs. In a later study (Fach *et al*., 2010) of the differential expression of cytokine transcription in neonatal and adult ovine alveolar macrophages following BRSV infection, the same authors found that peak mRNA levels of IL-1β and IL-8 in neonatal alveolar macrophages were several fold higher than levels induced in adult alveolar macrophages.

It is well known that infection of neonatal lambs with HRSV and BRSV results in pathology resembling infection in human infants (Olivier *et al*., 2011). The neonatal lamb model used here offers multiple advantages over the rodent model for studying the natural progression of HRSV disease and for investigating intervention strategies in natural and experimental BRSV infection. The specific aims of the present study were: (1) to characterize the immunohistochemical expression of IL-1β, IL-4, TNF-α, IFN-γ, IL-6, IL-8, IL-10 and IL-12 in the lungs of neonatal lambs infected experimentally with BRSV; (2) to compare cytokine expression patterns in airways with those of lung lesions occurring at different times post infection; and (3) to measure, using an enzyme-linked immunosorbent assay (ELISA), cytokine concentrations in lung tissue extracts from control and BRSV-infected animals.

Materials and Methods

Animals and Experimental Procedure

Thirty neonatal Merino lambs (2 days of age) purchased from the University of Extremadura Veterinary Faculty Farm. Absence of BRSV infection was confirmed by polymerase chain reaction (PCR). Commercial indirect ELISA kits with antigen-coated microtitre plates were used for detection of antibodies to bovine viral diarrhoea virus (BVDV), bovine herpesvirus (BHV)-1, BRSV, parainfluenza virus-3 and adenovirus-3 in serum, according to the manufacturer's instructions (Table 1). An indirect ELISA was used to determine serum antibody response to *Mannheimia haemolytica* (Mh) leukotoxin (van Rensburg *et al*., 2006). The lambs were free of Mh, BVDV, BHV-1, BRSV, parainfluenza-3 and adenovirus-3 based on serological testing.

Lambs were assigned randomly to two groups: infected (numbers 1–15) and control (numbers 16–30). The groups were kept in separate climate-controlled isolation rooms until they were killed. Lambs received ceftiofur sodium (Naxcel, 2.2 mg/kg once daily by intramuscular injection) to prevent secondary bacterial complications (Viuff *et al*., 2002; Fach *et al*., 2007) and were bottle-fed three times a day with commercial milk replacer. Lambs received 10% of their body weight in colostrum during the first 24 h of life.