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Marked induction of IL-6, haptoglobin and IFNγ following experimental BRSV infection in young calves

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Abstract

Bovine respiratory syncytial virus (BRSV) has been identified worldwide as an important pathogen associated with acute respiratory disease in calves. An infection model has been developed reflecting accurately the clinical course and the development of pathological signs during a natural BRSV-infection. In the experiments described in the present study, calves were infected at 13–21 weeks of age and reinfected 14 weeks later. Blood samples from the entire infection period were analysed for acute phase protein (haptoglobin) by ELISA and for expression (mRNA level in peripheral blood mononuclear cells) of the cytokines interleukin-2 (IL-2), interleukin-4 (IL-4), interleukin-6 (IL-6) and interferon-γ (IFNγ) by quantitative real-time reverse transcribed polymerase chain reaction (RT-PCR). IFNγ, interleukin-6 and haptoglobin were markedly induced together with development of clinical signs in response to the first infection with BRSV. The IFNγ response was biphasic, with an early peak at day 1–3 post infection (p.i.) and a later increase between day 5 and 8 p.i. Reinfection also resulted in an induction of IFNγ, but without induction of clinical signs, IL-6 and haptoglobin. These results indicate that early mediators connected with the innate responses are induced on a first encounter with the pathogen, but not on a second encounter (reinfection) where the adaptive immune system may act as the first line defence.

Keywords: Bovine respiratory syncytial virus; Experimental infection model; Cytokines; Acute phase proteins

1. Introduction

Bovine respiratory syncytial virus (BRSV) has been identified worldwide as an important pathogen associated with acute respiratory disease in calves (Larsen, 2000; Morrison et al., 1999). BRSV infections mainly occur as annual winter outbreaks affecting young calves during the first 6–9 months of life. The virus infects respiratory epithelium in both the upper and lower respiratory tract (Morrison et al., 1999; Viuff et al., 1996, 2002). The clinical response

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to BRSV varies from sub-clinical to severe, acute pneumonia (Kimman et al., 1988). The virus first replicates in the epithelial cells in the upper part of the respiratory tract, and later, around day 6 post infection (p.i.), in the lower respiratory tract (Viuff et al., 2002). Live BRSV and virus antigen decrease from day 7 to 8 p.i. and can scarcely be detected on day 15 p.i.. Enhanced respiratory rate and coughing can persist for longer time, probably due to lung damage or secondary bacterial infections (Tjornehoj et al., 2003). A strong and reproducible acute phase response has been shown peaking around day 7–8 p.i. (Heegaard et al., 2000).

Bovine RSV resembles human RSV (HRSV), which causes a similar disease in infants (Morrison et al., 1999). The immunology and pathogenesis of the RSV infection is not fully understood, but available evidence indicate that antibody and T-cell responses are both involved (Bembridge et al., 1998; Gaddum et al., 1996a,b; Larsen, 2000; Thomas et al., 1996). Cell-mediated immunity appears to be important for recovery, as children with defects in cell-mediated immunity develop more severe and persistent infection (Hall et al., 1986). The cytotoxic T-lymphocyte response also seems to be important in calves, as it is found that depletion of cytotoxic T-cells (CD8+) before experimental BRSV infection resulted in prolonged nasal shedding of virus and more extensive replication of virus in the lungs (Gaddum et al., 1996a; Taylor et al., 1995).

Cytokines may be one of the key factors determining the outcome of the infection, and both Th1-type and Th2-type polarized cytokine responses have been reported (Janeway, 2001; McInnes et al., 1998; Rontved et al., 2000). The Th1-type has been linked to a primarily cellular response and the Th2-type to an antibody response, although the Th1 cells also stimulate moderate levels of antibody production. In previous studies of animals infected with BRSV, cytokine expression has been studied by semi-quantitative reverse transcribed polymerase chain reaction (RT-PCR) on mononuclear cells isolated from the lung and blood from six experimentally infected gnotobiotic calves, which exhibited mild clinical signs (McInnes et al., 1998). Cytokines were analysed on day 7 p.i. and IFNγ, IL-2, IL-4 and IL-10 expression could be detected, indicating a mixed Th1/Th2-type of response (McInnes et al., 1998). Another experimental infection study demonstrated that BRSV induced a Th2-type response, with production of BRSV-specific IgE (Gershwin et al., 2000). Significant quantities of TNFα were found in the lungs of calves coinciding with occurrence of severe lung lesions and clinical signs at day 7 p.i. (Rontved et al., 2000), indicating that TNFα could play a role in the pathogenesis of BRSV in the lung.

An experimental model for BRSV infection in calves has previously been developed, which leads to clinical disease resembling outbreaks of BRSV-related pneumonia in naturally infected calves (Larsen et al., 1999; Rontved et al., 2000; Tjornehoj et al., 2003). In the present study, this model was used for further studies of the cytokine responses to BRSV infection and reinfection, measuring cytokine expression in blood mononuclear cells by quantitative real-time RT-PCR. In addition, we also analysed the serum concentration of the acute phase protein, haptoglobin in the entire infection and reinfection period.

All of four infected animals developed clinical signs with elevated body temperature and respiratory distress after the primary infection. Following the reinfection given 14 weeks later none of the previously infected animal showed any clinical signs. Expression of IL-6, haptoglobin and IFNγ were markedly induced as a response to the first experimental infection with BRSV, while reinfection resulted in a smaller induction of IFNγ and no induction of IL-6 and haptoglobin.

These results indicate that both inflammatory cytokines, acute phase proteins and Th1 cytokines are induced early after BRSV infection, and that the Th1 cytokine IFNγ is induced after reinfection, where animals show no clinical signs and no inflammatory response.

2. Materials and methods

2.1. Animals and experimental infections

Six 4–10 days old male Jersey calves were purchased from closed herds and reared in isolation units following normal management procedures for calves. The calves no. 1023, 1026, 1888 and 1892