Veterinary Immunology and Immunopathology 168 (2015) 159–163

Contents lists available at ScienceDirect

Veterinary Immunology and Immunopathology

journal homepage: www.elsevier.com/locate/vetimm

Short communication

Kinetics of pro-inflammatory cytokines, interleukin-10, and virus neutralising antibodies during acute ephemeral fever virus infections in Brahman cattle

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ARTICLE INFO

Article history:
Received 22 April 2015
Received in revised form 7 September 2015
Accepted 9 September 2015

Keywords:
Bovine ephemeral fever
Cytokines
Fever
Humoral immunity
Innate immunity

ABSTRACT

While fever and inflammation are hallmark features of bovine ephemeral fever (BEF), the cytokine networks that underlie the acute phase of the disease have not been empirically defined in cattle. This study characterised the plasma kinetics of proinflammatory cytokines (IL-1β, IL-6, TNF-α) and IL-10 during acute BEF and elucidated on the relationship between the onset of the virus neutralizing antibody response and resolution of viraemia in natural BEF virus (BEFV) infections in cattle. Plasma from three BEFV-infected and three uninfected cattle was tested for the study cytokines by a cELISA, viraemia monitored by qRT-PCR, and virus neutralizing antibody titres determined using a standard protocol. Unlike the negative controls, plasma concentrations of IL-1β, TNF-α, IL-6, and IL-10 were consistently increased in the three virus-infected animals. Two of the infected heifers were recumbent and pyrexic on the first day of monitoring and increased cytokine production was already in progress by the time viraemia was detected in all the three infected animals. In all the virus-infected heifers, IL-1β was the most strongly expressed cytokine, IL-6 and IL-10 manifested intermediate plasma concentrations while TNF-α was the least expressed and demonstrated bi-phasic peaks three and five days after the onset of pyrexia. In two of the BEFV-infected heifers, viraemia resolved on the day of seroconversion while in the other infected animal, viral RNA was detectable up to three days after seroconversion. The present data document variable increase in plasma IL-1β, IL-6, TNF-α, and IL-10 during natural BEFV infections and the fact that upregulation of all but TNF-α precedes seroconversion. In addition to virus neutralising antibodies, it is likely that cytokine-mediated cellular mechanisms may be required for resolution of viraemia in BEF. Considering the anti-inflammatory properties of IL-10, its upregulation may potentially antagonise the fever response in BEFV-infected cattle.

1. Introduction

Bovine ephemeral fever (BEF) is a vector-borne disease of cattle that is prevalent in tropical and subtropical regions of Asia, Australia, and Africa (St George et al., 1995). It is caused by a single stranded RNA virus classified under the genus Ephemerovirus within the family Rhabdoviridae. In some but not all affected cattle, the disease is characterised by a transient polyphasic fever, inappetance, synovitis, muscle stiffness, lameness, and paresis and/or paralysis (Basson et al., 1970; Young and Spradbrow, 1990; St George et al., 1995). Clinically sick animals also often exhibit biochemical blood dyscrasias including but not limited to hypocalcaemia and hyperfibrinogenaemia (St George et al., 1984; Young and Spradbrow, 1990).

Several studies have indicated that inflammation and fever are hallmark features of acute BEF (Basson et al., 1970; Young and Spradbrow, 1990). Pathological abnormalities alluding to the inflammatory nature of the disease include but are not limited to neutrophilia, polyserositis, vasculitis, tenovaginitis, and fibrinous exudates within the pleural, peritoneal, pericardial and joint cavities (Basson et al., 1970). As BEFV is not cytolytic (Young and Spradbrow, 1990), pro-inflammatory cytokines are believed to play a significant role in the pathogenesis and clinical expression of the acute disease (St George et al., 1995; Uren et al., 1989). In studies done in Australia, treatment of BEFV-infected cattle with the anti-inflammatory drug phenylbutazone abrogated fever (Uren et al., 1989). In a conference report by Uren and Zakrzewski (1989), increased expression of plasma IL-1β and TNF-α were reported in febrile cattle experimentally infected with BEFV. While that report

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http://dx.doi.org/10.1016/j.vetimm.2015.09.004
0165-2427/

Table 1
Primer and probes used in the qRT-PCR assay for the detection of BEFV RNA in the present study (Lew et al., 2006).

Name	Sequence 5′ → 3′	Final conc.
BEFVgF2	TTT TAT CWG CTG TTG TAG GTT GGT	800 nM
BEFVgR2	AAC AGC CCA AAT TGT CCA TCT T	800 nM
BEFVgMGB (TaqMan® Probe)	6FAM-ACG GCA AAG GCA G-MGBNFG	100 nM

alluded to the potential role of pro-inflammatory cytokines in the pathogenesis of BEF, conclusive studies have not been done in adult cattle to corroborate the findings. In addition, while post-infection/vaccination immunity in BEF has been attributed to virus neutralizing antibodies (Aziz-Boaron et al., 2013), the contributory role of cellular immune mechanisms has not been investigated. In particular, the role(s) played by cytokines in driving the innate-adaptive immune response transition also remain unstudied in BEF.

This research was conducted to describe the plasma kinetics of IL-1β, IL-6, IL-10, and TNF-α during acute BEF and to assess whether increased cytokine production is temporally associated with fever in adult cattle naturally infected with BEFV. The study further assessed the relationship between the onset of virus neutralising antibodies and resolution of viraemia.

2. Materials and methods

2.1. Study cattle

The six-day clinical monitoring and sample collection periods were initiated as soon as the index BEF case was confirmed by positive qRT-PCR. Only six out of 24 sentinel cattle studied during a BEF outbreak, including three BEFV-stricken and three uninfected heifers, met the study inclusion criteria. All the six animals included in the cytokine study were seronegative to regionally prevalent arboviruses of animal health significance including Akabane, bluetongue, and epizootic haemorrhagic viruses. The remaining 18 animals were excluded from the study after being found seropositive for at least one of these viruses.

2.2. qRT-PCR

The qRT-PCR protocol used for BEFV detection in the present study was previously standardised by Lew et al. (2006). The viral RNA was extracted from EDTA blood samples using the MagMAX-96 viral RNA isolation kit (Applied Biosystems, CA, USA), eluted with 50 μl elution buffer, and stored at −80 °C until testing. The qRT-PCR assay was optimized with the AgPath-ID One-Step RT-PCR Kit (Applied Biosystems, CA, U.S.A.) using a total volume of 25 μl. Briefly, a master mix consisting of 4.0 μl nuclease-free water, 12.5 μl 2× RT-PCR buffer, 1.0 μl 25× RT-PCR enzyme mix, 1.0 μl BEFVgF2 forward primer (10 μM), 1.0 μl BEFVgR2 reverse primer (10 μM) and 0.5 μl BEFVgMGB (TaqMan®) Probe (5 μM) for one reaction was prepared and 5 μl RNA template was added (refer to Table 1 for the assay primers and probes). For amplification the following temperature profile was used: 30 min at 50 °C (reverse transcription), 10 min at 95 °C (inactivation reverse transcriptase/activation Taq polymerase), followed by 50 cycles of 15 s at 95 °C (denaturation), 60 s at 60 °C (annealing and elongation). To verify the correctness of the nucleic acid extraction and/or the qRT-PCR reaction, a positive and a negative extraction control, and positive and negative reverse transcription and amplification controls were included.

Table 2
Rectal temperatures (°C) of the six study cattle during the six-day monitoring period. Febrile temperatures are denoted with an asterisk.

Days						
	1	2	3	4	5	6
Bovine ephemeral fever virus-infected ($n = 3$)						
B113	39.1*	38.6	38.9	38.7	38.8	38.5
B118	41.0*	38.2	38.1	38.4	37.9	38.4
B121	38.7	38.6	39.3	38.7	38.2	38.5
Uninfected negative controls ($n = 3$)						
B101	37.8	38.5	38	37.7	38.7	38.3
B114	38.6	39.0	38.5	38.5	38.6	38.6
B115	38.1	38.5	38.3	38.3	38.5	38.5

2.3. Virus neutralisation test

The modified version of the virus neutralisation test (Uren et al., 1994) applied in the present study involved testing of preheated serum samples in 96-well plates containing Minimum Essential Medium or MEM (Sigma-Aldrich, St. Louis, MO, USA) supplemented with 10% foetal bovine serum. Briefly, 50 μl of serially diluted preheated sera held in quadruplicate wells were incubated with 50 μl of MEM containing 100 TCID50 of BEFV for 1 h at 37 °C. Then, 100 μl of MEM containing BSR cells at 2×10^5 cells per ml was added and the plates read for cytopathic effect after incubation at 37 °C for five days (Sato et al., 1975). The virus neutralising antibody titres were calculated using the 50% end point method of Reed and Muench (1938).

2.4. Immunoenzymatic assays for bovine cytokines

The cytokine (IL-1β, IL-6, IL-10, and TNF-α) assays were performed using Cusabio® competitive inhibition ELISA according to the kit manual instructions and the ELISA plates read at 450 nm. Using the "Curve Expert 1.3" software program (Hyams Development, AL, USA), the protein standards OD_{450} readings were used to generate a standard curve which was then used to derive the plasma cytokine concentrations as specified in the Cusabio® competitive inhibition ELISA kit instructions.

3. Results and discussion

3.1. Fever, seroconversion, and resolution of viraemia

On day 1, BEFV-infected animals No B113 and B118 were clinically ill, recumbent, and had rectal temperatures (recT) of 39.1 and 41.0 °C respectively (Table 2). By day 2, however, the fever had subsided (Table 2); the transient nature of these clinical signs was consistent with classic BEF in two of the virus-infected heifers. As the mean incubation period in BEF is about three to five days, it is probable the first fever peak in the two febrile heifers occurred prior to day 1. The other focus of this study was to evaluate whether neutralising antibodies were the only important factor in the resolution of viraemia during acute BEF. Vanselow et al. (1985) reported that virus neutralizing antibody titres ≥45 were associated with immunoprotection against BEF. According to our results, however, it is apparent that virus neutralisation in BEF may occur over a wider antibody titre range than previously suggested. In the present study, viraemia was observed for two days in the two febrile heifers and promptly resolved when the neutralizing antibody titres reached 8 and 16, respectively (Table 3). In the other virus-infected animal, however, viraemia continued for three days after seroconversion and only resolved when the antibody titre reached 72 (Table 3). While these observations underscore