Veterinary Microbiology 186 (2016) 111–116

Contents lists available at ScienceDirect

Veterinary Microbiology

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

Short communication

Kinetics of selected plasma cytokines during innate-adaptive immune response transition in adult cattle infected with the bovine ephemeral fever virus

R. Barigye*, L.F. Melville, S. Davis, S. Walsh, N. Hunt, R. Hunt

Berrimah Veterinary Laboratories, Department of Primary Industry & Fisheries, GPO Box 3000, Darwin, NT 0801, Australia

ARTICLE INFO

Article history:
Received 6 January 2016
Received in revised form 12 February 2016
Accepted 23 February 2016

Keywords:
Bovine ephemeral fever
Cytokines
Innate immunity
Adaptive immunity

ABSTRACT

While virus neutralizing antibodies are known to be variably protective against bovine ephemeral fever (BEF) virus (BEFV) infections, the cytokine events that mediate the nascent adaptive immune response have not been defined in cattle. This study determined the plasma kinetics of IL-2, IFN-γ, IL-6, and IL-10 during the period of innate-immune response transition and evaluated the relationship between the virus neutralizing antibody response and viraemia in BEFV-infected cattle. Plasma from four virus-infected and uninfected negative control animals was tested by cytokine-specific immunoenzymatic assays, viraemia monitored by qRT-PCR, and virus neutralizing antibody titres determined using a standard protocol. Unlike the negative controls, plasma IL-6 and IL-10 were increased in all the virus-infected animals starting several days prior to initiation of viraemia. In one animal, plasma IL-2 and IFN-γ were consistently higher than in the other three virus-infected animals and the negative control mean. The animal with the strongest IL-2 and IFN-γ responses had the shortest viraemia while the heifer with the lowest IL-2/IFN-γ indices demonstrated the longest viraemia. Evidently, increase in plasma IL-6 and IL-10 precedes seroconversion during BEFV infections in cattle suggesting the two cytokines may influence immunological events that pave way to B-cell activation and seroconversion. While there is remarkable variability in IL-2 and IFN-γ expression amongst BEFV-infected animals, increased plasma levels of the two cytokines appear to be associated with a shorter viraemia. Ongoing studies will help define the precise role of T cells in anti-BEFV adaptive immune responses.

© 2016 Published by Elsevier B.V.

1. Introduction

Bovine ephemeral fever (BEF) is an insect-borne viral disease of cattle caused by a single stranded RNA virus that belongs to the genus *Ephemerovirus* within the family *Rhabdoviridae*. The disease has been reported in tropical and subtropical regions of Asia, Australia and Africa but not in North America or Europe (St. George, 1986). Both natural bovine ephemeral fever virus (BEFV) infections and vaccination with BEFV-derived immunogens may be followed by solid immunity that is attributed to production of virus neutralizing antibodies (Uren et al., 1994; Aziz-Boaron et al., 2013). However, in a number of studies that specifically investigated the immunoprotection conferred by inactivated BEFV vaccines in cattle, several animals succumbed to challenge infection despite having developed high neutralizing antibody titres against the virus (Tzipori and Spradbrow, 1973; Della-Porta and Snowdon, 1979; Aziz-Boaron et al., 2014). Considering findings made in these studies, it is plausible that adaptive immunity in BEF is likely to involve cellular mechanisms that arguably augment the virus neutralizing antibody response. While this is highly likely, however, such a contributory role of cell mediated immune mechanisms in anti-BEFV immunity is still poorly understood. In a conference report by Uren et al. (1993), T-cell lines established in vitro from peripheral blood mononuclear cells taken from cattle immunised with a subunit BEFV immunogen proliferated in presence of native BEFV antigens (Uren et al., 1993). Interestingly, increased IL-2 levels were demonstrated in supernatants of the T-cell cultures alluding to presumed cellular events that arguably may precede seroconversion in BEF. While these findings provided anecdotal evidence for a possible role of T-cell mechanisms in immune responses against BEFV (Uren et al., 1993), however, the presumed in vivo cellular mechanisms that characterise the

* Corresponding author.
E-mail address: rbarigye@yahoo.com (R. Barigye).

http://dx.doi.org/10.1016/j.vetmic.2016.02.024
0378-1135/© 2016 Published by Elsevier B.V.

nascent adaptive immune response in BEF in cattle remain largely unstudied.

The present study was therefore done to describe the kinetics of Th1 indicator cytokines (IL-2 and IFN-γ), IL-6, and IL-10 during the innate-immune response transition in adult cattle infected with the BEFV. The study also evaluated the relationship between the kinetics of the virus neutralizing antibody response and the duration of viraemia.

2. Materials and methods

2.1. Study cattle

Blood and serum samples were collected from a group of 18 adult sentinel cattle during an ongoing BEF outbreak at Beatrice Hill Research Farm in Northern Territory, Australia. The eight heifers that met the inclusion criteria included four animals that tested qRT-PCR-positive for BEFV plus four uninfected heifers that served as the negative controls. Plasma samples collected beginning three days prior to the detection of viraemia through to the ninth day of the study were tested for plasma cytokines including IL-2, IFN-γ, IL-6 and IL-10. At the beginning of the study, all the eight animals were seronegative to regionally important arboviruses of animal health significance namely Akabane, bluetongue, and epizootic haemorrhagic viruses. Note that samples from the other 10 animals were excluded from the study after being found seropositive for at least one of the regionally endemic arboviruses.

2.2. qRT-PCR assay for bovine ephemeral fever virus

Viral RNA was extracted from EDTA blood samples using the MagMAX-96 viral RNA isolation kit (Applied Biosystems, CA, U.S.A) and subsequently tested for BEFV using a protocol previously standardised by Lew et al. (2006) with minor in-house modifications as recently reported (Barigye et al., 2015). A positive and a negative extraction control, and positive and negative reverse transcription and amplification controls were included in the assay to verify the correctness of the nucleic acid extraction and/or the qRT-PCR reaction. The primers and concentrations used in this study are indicated in Table 1.

2.3. Virus neutralizing antibody test

Preheated serum samples were tested using a modified version of the virus neutralization test initially reported by Uren et al. (1994). Fifty μl of serially diluted sera were held in quadruplicate wells of 96 well plates and incubated with 50 μl of Minimum Essential Medium (MEM) containing 100 TCID50 of BEFV for 1 h at 37 °C. One hundred μl of MEM containing 2×10^5 BSR cells (a clone of baby hamster kidney cells) per ml were added and the plates read for cytopathic effect after incubating 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 plasma samples collected beginning three days prior to the detection of viraemia through to the ninth day of the study were tested for plasma cytokines including IL-2, IFN-γ, IL-6 and IL-10. The assays for IL-6 and IL-10 were done using Cusabio® competitive inhibition ELISA according to the kit manual instructions. Using the "Curve Expert 1.3" software program (Hyams Development, AL, USA), the OD_{450} readings for the protein standards were plotted and a standard curve generated. The plasma concentrations of IL-6 and IL-10 in the test samples were derived by extrapolation of the respective OD_{450} readings onto the standard curve. In the case of IL-2 and IFN-γ, plasma samples were tested by the Cusabio® antigen capture ELISA according to the kit manual instructions. As the IL-2 and IFN-γ concentrations in some of the samples were slightly out of the detection limit of the standard curve, plasma levels of the two cytokines were inferred from the magnitude of the OD_{450nm} readings.

3. Results

3.1. Viraemia and kinetics of the virus neutralising antibody response

In all the four BEFV-infected heifers, viraemia was first detected on day 4 and lasted only three days in animal B20, four days in animals B05 and B10, and six days in heifer No B11 (Table 2). With the exception of animal B11, it is noteworthy that in animals B05, B10 and B20, the viraemia resolved a day after seroconversion when the neutralising antibody titres were 64, 144 and 72, respectively. On the other hand, the viraemia in animal B11 continued for six days and only resolved after the virus neutralising antibody titre reached 144 (Table 2). In animal B05, following cessation of viraemia, the antibody response continued to rise, peaked around day 13, before starting to show a gradual decline until day 15 when study observations where were concluded. In animal B10, the antibody response peaked on day 11 and thereafter started declining while showing some minor antibody titre fluctuations. In animal B11 the antibody response peaked on day 12 and thereafter started declining. In animal B20, the antibody response peaked on day 8 before showing a gradual decline with characteristic daily fluctuations.

3.2. Kinetics of plasma IL-10, IL-6, IL-2, IFN-γ

During the nine days of cytokine screening, plasma IL-10 values for the individual virus-infected animals were consistently greater than for the negative controls (Fig. 1A). Interestingly, the increase in plasma IL-10 was already noticeable on day 1 which was three days prior to the detection of viraemia in all the four animals. When the cytokine production trends were evaluated, the plasma IL-10 concentrations for animal B05 were slightly higher than for the other three virus infected heifers for the larger part of the nine days with only very few exceptions. In respect to plasma IL-6 concentrations, the individual animal values were also generally greater in virus-infected heifers than in the negative controls and seemed to pick momentum around day 3 which was a day prior to the detection of viraemia.

Table 1
Primer and probes used in the BEFV-qRT-PCR assay in the present study.

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

Download English Version:

https://daneshyari.com/en/article/5799733

Download Persian Version:

https://daneshyari.com/article/5799733

Daneshyari.com