Veterinary Microbiology 150 (2011) 270–277

Contents lists available at ScienceDirect

Veterinary Microbiology

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

Characterization of ovine herpesvirus 2-induced malignant catarrhal fever in rabbits

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ARTICLE INFO

Article history:
Received 10 November 2010
Received in revised form 26 January 2011
Accepted 14 February 2011

Key words:
Malignant catarrhal fever
Ovine herpesvirus 2
Rabbit model
Lymphoproliferation
Gene expression

ABSTRACT

Malignant catarrhal fever (MCF) is a frequently fatal lymphoproliferative disease syndrome primarily of ruminant species, caused by gammaherpesviruses in the genus *Macavirus*. Ovine herpesvirus 2 (OvHV-2), carried by sheep, causes sheep-associated MCF worldwide, while Alcelaphine herpesvirus 1 (AlHV-1), carried by wildebeest, causes wildebeest-associated MCF, mainly in Africa. Diseases in rabbits can be induced by both viruses, which are clinically and pathologically similar; however, recent studies revealed different expression of viral genes associated with latency or lytic replication during clinical disease between the two viruses. In this study, we further characterized experimentally induced MCF in rabbits by nebulization with OvHV-2 from sheep nasal secretions to elucidate the course of viral replication, along with in vivo incorporation of 5-Bromo-2′-Deoxyuridine (BrdU), to evaluate lymphoproliferation. All six rabbits nebulized with OvHV-2 developed MCF between 24 and 29 days post infection. OvHV-2 DNA levels in peripheral blood leukocytes (PBL) remained undetectable during the incubation period and increased dramatically a few days before onset of clinical signs. During the clinical stage, we found that predominantly lytic gene expression was detected in PBL and tissues, and both T and B cells were proliferating. The data showed that the viral gene expression profile and lymphoproliferation in rabbits with OvHV-2 induced MCF were different from that in rabbits with AlHV-1 induced MCF, suggesting that OvHV-2 and AlHV-1 may play a different role in MCF pathogenesis.

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1. Introduction

Malignant catarrhal fever is a frequently fatal, lymphoproliferative disease syndrome of many species in the Artiodactyla order, including cattle, bison, and deer, as well as pigs (Russell et al., 2009), caused by gammaherpesviruses in the genus *Macavirus* (Davison et al., 2009). Although several viruses in the MCF virus group are capable of inducing the disease (Li et al., 2005), the most important members in this group are Alcelaphine herpesvirus 1 (AlHV-1) and ovine herpesvirus 2 (OvHV-2) (Russell et al., 2009). AlHV-1 is carried by blue and black wildebeest and the induced disease is called wildebeest-associated MCF (WA-MCF) (Plowright, 1990). OvHV-2 is carried by sheep worldwide and the disease is called sheep-associated MCF (SA-MCF) (Plowright, 1990). WA-MCF is an important economic concern to cattle populations in parts of Africa where they share grazing with wildebeest (Bedelian et al., 2007), and has also caused losses in zoological collections and game farms where wildebeest

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0378-1135/$ – see front matter. Published by Elsevier B.V.
doi:10.1016/j.vetmic.2011.02.016

are present (Blake et al., 1990; Meteyer et al., 1989). SA-MCF is the leading cause of death of ranched American bison and has been associated with numerous outbreaks due to their high susceptibility (Li et al., 2006; Schultheiss et al., 2000). SA-MCF is also economically important in other farmed ruminant species including Bali cattle in Indonesia (Daniels et al., 1988; Wiyono et al., 1994) and red deer in New Zealand (Audige et al., 1994; Orr and Mackintosh, 1988), as well as causing sporadic problems for many domestic and wild ruminants worldwide (Heuschele, 1988). Rabbits develop an MCF-like disease following experimental infection with either OvHV-2 or AlHV-1 (Buxton and Reid, 1980) and are used as an animal model to study AlHV-1 induced MCF pathogenesis (Dewals et al., 2008). Experimentally induced MCF in rabbits with OvHV-2 by nebulization was successful (Gailbreath et al., 2008) and its usefulness as a model that involves a natural mode of transmission to study certain features of infection and disease has been under investigation, which is the purpose of this study.

A striking feature of MCF is the species-specific nature of the disease. Generally, clinical disease in susceptible species is associated with some combination of lymphoid hyperplasia, arteritis–phlebitis, abomasitis (or gastritis), enteritis–colitis–typhilitis, panophthalmitis, cystitis, hepatitis, and encephalitis (Liggitt and DeMartini, 1980a,b). American bison with natural or experimentally induced MCF due to OvHV-2 generally have modest lesions in lymph nodes and blood vessels (O'Toole et al., 2007). The most consistent and severe lesions in bison are inflammation and ulceration in the digestive tract, particularly in the oral cavity, esophagus, forestomachs, abomasum, cecum and colon. Hemorrhagic cystitis is almost invariably found in bison when clinical signs develop. Urothelium in the renal pelvis, ureter and urethra is degenerated and/or sloughed (O'Toole et al., 2002, 2007). Necrotizing arteritis, which is such a characteristic feature of MCF in many ruminant species, is inconspicuous.

The pathogenesis of MCF induced by either AlHV-1 or OvHV-2 is not well understood. Although both viruses are closely related antigenically and genetically, causing a similar disease syndrome and lesions (Plowright, 1990), increasing evidence indicates that there are significant differences between OvHV-2 and AlHV-1 in several aspects, including infection and shedding from natural hosts, the requirements for in vitro propagation, and expression of viral genes responsible for lytic replication in clinically affected hosts. Shedding patterns differ between natural hosts: infected sheep shed the virus sporadically with a short-lived episode, more frequently between 6 and 9 months of age (Li et al., 2004), and newborn lambs are not the source of infection (Li et al., 2004). By contrast, most newborn wildebeest calves are infected and shed virus continuously until 3–4 months of age, and are the primary virus source for transmission (Mushi et al., 1981). Cell tropisms are different between the two viruses: AlHV-1 readily grows in various cell lines (Plowright, 1990) and infection can be induced in cattle or rabbits by cell-free virus through various routes, including intranasally, intramuscularly and intravenously (Haig et al., 2008; Mushi, 1980). In contrast, OvHV-2 has not been propagated in vitro yet, even in cell lines that can support AlHV-1 in vitro propagation. Cell-free OvHV-2 from sheep nasal secretions failed to induce infection in sheep following intravenous or intraperitoneal inoculation (Li et al., 2008). Recent studies revealed that the transcripts of the open reading frame (ORF) 25, a gene encoding the major capsid protein, were present in virtually all the tissues of cattle, bison (Cunha et al., 2007) and rabbits (Gailbreath et al., 2008) with OvHV-2-induced MCF, but not present in the tissues (spleen and lymph nodes) of rabbits with AlHV-1-induced MCF (Dewals et al., 2008), suggesting that viral infection at the tissue level in clinically susceptible hosts may also be different. In this study, we experimentally induced MCF in rabbits by intranasal inoculation of OvHV-2 from sheep nasal secretions to determine: (1) if rabbits can be a reliable model for OvHV-2-induced MCF; (2) viral replication status based on viral gene expression; and (3) lymphoproliferation based on in vivo incorporation of 5-Bromo-2′-Deoxyuridine (BrdU).

2. Materials and methods

2.1. Rabbits and experimental infection

A total of 10 four-month-old New Zealand white rabbits were obtained from the Western Rabbits Company of Oregon and maintained at Washington State University, Pullman, WA in accordance with an approved animal care and use protocol. The rabbits were individually caged and held in the same isolation room. After an acclimation period of one week, each rabbit was given three consecutive daily doses of 5 mg/kg enrofloxacin (Baytril) by intramuscular injection into the caudal thigh muscles to prevent infection by potential bacterial pathogens present in the sheep nasal secretions. The rabbits were divided into two groups: a test group ($n = 6$) and a negative control group ($n = 4$). The nasal secretion inoculum used in this study was the same pooled inoculum used in a previous rabbit study (Gailbreath et al., 2008). Each rabbit in the test group was inoculated by nebulization with 2 ml of pooled sheep nasal secretions containing 10^7 OvHV-2 DNA copies using the same procedure described previously (Gailbreath et al., 2008). The rabbits in the negative control group each received 2 ml of nasal secretions collected from OvHV-2 negative sheep. The nebulization procedure was carried out in an operation room, which was separated from the rabbit holding room. The nebulization for the negative control group was carried out first, and then for the positive group. All the rabbits were observed daily, including monitoring body temperatures, and were euthanized within 48 h of the onset of pyrexia ($>104\,^{\circ}$F).

2.2. Sample collection and preparation

Two milliliters of EDTA-anticoagulated blood from an aural vein of each rabbit was collected twice a week. Blood samples were prepared for testing for MCF viral antibody by competitive ELISA (cELISA), OvHV-2 DNA by nested PCR, and incorporation of BrdU in lymphocytes (after 20 days post infection, DPI) by flow cytometry. Additional 20 ml blood samples were collected before euthanasia, and the buffy