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Viral load, organ distribution, histopathological lesions, and cytokine mRNA expression in goats infected with a molecular clone of the caprine arthritis encephalitis virus

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Abstract

Caprine arthritis encephalitis virus (CAEV) is a lentivirus of goats that causes persistent infection characterized by the appearance of inflammatory lesions in various organs. To define the sites of persistence, 5 goats were infected with a molecular clone of CAEV, and the viral load was monitored by real-time-PCR and RT-PCR in different sites 8 years after infection. The lymph nodes proved to be an important virus reservoir, with moderate virus replication relative to what is reported for lentiviruses of primates. Mammary gland and milk cells were preferred sites of viral replication. The viral load varied significantly between animals, which points to an important role of the genetic background. We found a clear association between occurrence of histopathological lesions and viral load in specific sites. The mRNA expression analysis of several cytokines did not reveal differences between animals that could explain the considerable individual variations in viral load observed.

Keywords: Caprine arthritis encephalitis virus; CAEV; Cytokine; MVV; Small ruminant lentiviruses; SRLV; Viral load; Real-time PCR; Lentivirus; Animal genetic

Introduction

Persistence of the infecting virus in various organs and tissues in spite of a robust immune response is a hallmark of all lentiviral infections. In the case of HIV, SIV, and FIV, the infections are associated with immunodeficiency that develops slowly over months to years. In contrast, no immunodeficiency is observed in infections caused by Maedi Visna (MVV) in sheep and caprine arthritis encephalitis virus (CAEV) in goats. Although these viruses were previously considered to be pathogens specific for sheep and goats, respectively, recent evidence indicates that they may be capable of infecting both species and in fact also some small ruminant species living in the wild (Leroux et al., 1997; Shah et al., 2004; Pisoni et al., 2005; Guiguen et al., 2000). For these reasons, MVV and CAEV are also referred to as "small ruminant lentiviruses (SRLV)" (Leroux et al., 1997; Peterhans et al., 2004). Depending on virus and host genetic factors, approximately one third of SRLV infected animals may develop clinical signs of disease such as arthritis, the most important clinical manifestation in goats, pneumonia, mastitis, and more rarely, encephalitis.

SRLV infect mainly cells of the monocyte/macrophage lineage and dendritic cells (Zink et al., 1990; Ryan et al., 2000) although, different from the immunodeficiency-causing lentiviruses, these viruses do not infect lymphocytes. Infection of kids via colostrum is the principal path of transmission, followed by horizontal spread via infected secretions (Peterhans et al., 2004; Blacklaws et al., 2004).

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Strong indirect evidence suggests that arthritic goats have a higher viral load compared to their asymptomatic counterparts. Arthritic animals have higher antibody titers especially to particular epitopes of the envelope glycoprotein (Knowles et al., 1990; McGuire et al., 1992; Bertoni et al., 1994). Virus RNA was detected by in situ hybridization exclusively in the joints of animals with chronic arthritis and not in organs of clinically healthy animals (Lechner et al., 1997c). Additionally, the failure to isolate virus or to detect provirus DNA or viral RNA by PCR and RT-PCR in peripheral blood mononuclear cells (PBMC) from numerous clinically healthy animals indicates that these animals may have a lower viral load (unpublished observations).

Thus, the situation may be similar to that observed in HIV infected long-term non-progressors or in patients treated with highly active antiretroviral therapy (HAART). In these persons, viral mRNA can be detected in PBMC only by extremely sensitive methods (Sharkey et al., 2000; Rodes et al., 2004). Several authors have assessed the persistence of HIV in different organ reservoirs (Chun and Fauci, 1999; Pierson et al., 2000; Gunthard et al., 2001) and followed the changes over time in the abundance of unspliced (US) and multiply spliced (MS) mRNA, thus demonstrating their significance as reliable markers of disease progression (Saksela et al., 1994; Lewin et al., 1999). An increased level of MS mRNA in PBMC was shown to correlate with the appearance in the blood of newly infected cells coming from tissue reservoirs. In the case of the feline lentivirus – feline immunodeficiency virus (FIV) – an increase of MS mRNA in PBMC has been associated with virus activation in persistently infected cats (Tomonaga et al., 1995).

Like HIV, SRLV show a sequential gene expression with a first transcription step involving the *rev* gene (MS mRNA) that leads, in a second step, to the expression of structural genes (US mRNA or singly spliced mRNA) (Vigne et al., 1987; Ravazzolo, 1993; Schoborg, 2002). Whether this two-step transcription is implicated in disease progression and virus persistence in infected goats is not known.

The principal objective of this work was to determine the viral load in selected sites and the mRNA expression patterns (MS versus US) in long-term CAEV infected goats. Additionally, we explored potential correlations between viral load and the extent of pathological changes in different target organs and possible influences of the viral load on the expression pattern of several cytokines in the draining lymph nodes.

Results

Virus isolation

Cells or tissue samples were co-cultivated with GSM cells, and the co-cultures were passaged 9 times with immunohistochemical staining performed at passages 3, 5, 7, and 9. Co-cultures showing no syncytia formation up to passage 9 and with negative immunohistochemical staining results were considered free of infectious virus. Virus isolation succeeded only in goats #13 and #4. The virus was isolated from milk cells (goat #4), alveolar macrophages, and PBMC (goats #4 and #13) co-cultivated with goat synovial membrane cells. Virus isolation failed for all the other animals (#3, #10, and #11) independently of the tissues examined.

CAE provirus quantification

The number of provirus copies was determined by comparing the Ct values obtained amplifying an *env* fragment in a particular sample to a standard curve, which also shows that the PCR reaction was very efficient and linear from 1 to 10^7 copies (Fig. 1). The provirus copy numbers differed between the five animals and the tissues analyzed (Table 2). The animals could be divided into two groups: goats #3, #10, and #11 with a low provirus load and goat #4 and #13 with a high provirus load. Provirus was detected in all the animals, confirming the persistent nature of SRLV infection. In the low provirus group (#3, #10, and #11), provirus was identified in the PBMC fraction and in at least two lymph nodes. Furthermore, provirus was detected in the mammary gland and milk cells of goat #3 and in the spleen of the males #10 and #11.

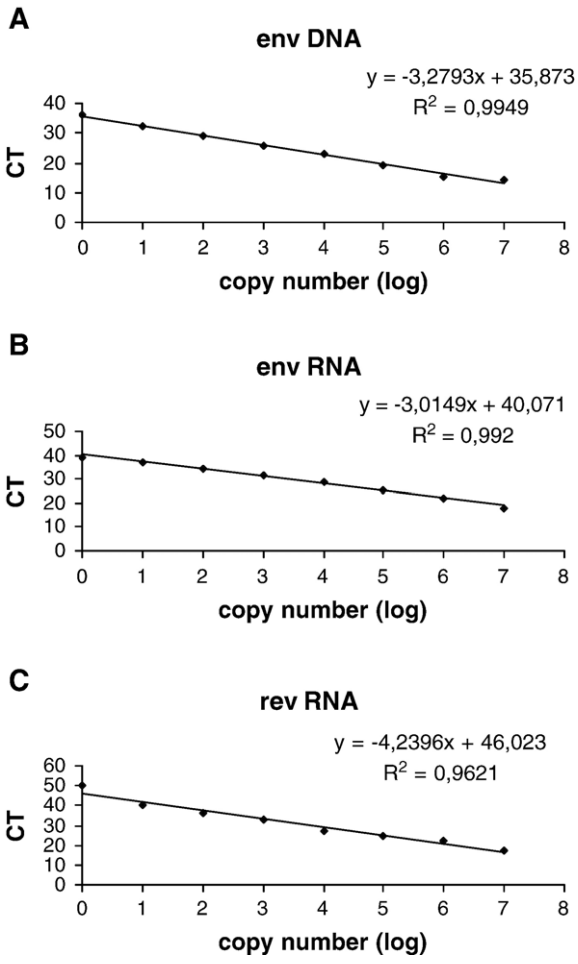

Fig. 1. Standard curves generated with known copy numbers of the *env* plasmid (A), the *env* transcripts (B), and the *rev* transcripts (C). 10^0 to 10^7 standard templates were amplified by real-time PCR (*env* plasmid) or RT real-time PCR (*env* and *rev* in vitro-transcribed RNA) to generate the standard curve permitting to calculate the copy number (x) present in biological samples according to the Ct value (y) obtained.