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Short communication

Phenotypic alteration of blood and milk leukocytes in goats naturally infected with caprine arthritis-encephalitis virus (CAEV)

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Abstract

Caprine arthritis-encephalitis virus (CAEV) causes a persistent and slow progressive infection in goats, characterized by chronic proliferative sinovitis, arthritis and, less frequently, pneumonia. Infected goats can also be affected by interstitial mastitis. The aim of this study was to evaluate the influence of CAEV infection on the phenotypic composition of leukocyte subsets in blood and milk, during lactation. CD8 positive cells in blood and milk were more numerous in CAEV positive goats when compared to negative goats. γδTcR positive cells were higher in blood but not in milk of CAEV positive goats. The content of cells expressing MHC class II molecules was higher in blood from CAEV negative goats, while the content of activated cells was higher in milk from CAEV infected goats.

Keywords: Leukocytes; CAEV; Goat; Flow cytometry; Milk

1. Introduction

Caprine arthritis-encephalitis virus (CAEV) is a non-oncogenic retrovirus, belonging to the *Lentivirus* genus, that causes a persistent and slowly progressive degenerative inflammatory disease in the host (Narayan and Cork, 1985). After variable and prolonged incubation periods, lasting months to years, CAEV-infected adult goats develop clinical signs mainly characterized by chronic proliferative synovitis and periarthritis, and, less frequently, pneumonia. In kids of 2–4 months of age, leuko-encephalomyelitis is observed. CAEV is mainly macrophage-tropic (Gorrel et al., 1992): viral replication in macrophages of target tissues produces inflammatory type lesions. Nevertheless, other cell types, such as fibroblasts, acinar epithelial cells and endothelial cells are susceptible to *in vivo* infection and are likely to contribute to lesion development (Carrozza et al., 2003; Sanna et al., 1999). While opportunistic infections and immune deficiency are not characteristic features of CAEV infection, only a few studies on possible effects of viral infection on the composition of blood leucocytes have been undertaken, but no definitive results

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are available. Some researchers report no changes in the composition of blood leukocytes of infected animals (Wilkerson et al., 1995a,b; Grezel et al., 1997). Others reported a drop in the percentage of CD4 and CD8 positive blood lymphocytes, and an increase of γδ T and B positive lymphocytes, that was related to recurrent lymphocytosis, probably due to continuous antigen presentation (Mdurvwa et al., 1994; Jolly et al., 1997; Perry et al., 1995). The virus also causes lesions in the mammary gland or interstitial mastitis (Lerondelle et al., 1989); in fact it should be frequently detected in macrophages, and other mammary cell types (Le Jan et al., 2005). Therefore, consumption of colostrum and milk by neonates and contamination of lactating goats during milking are responsible for transmission of CAEV (Zink et al., 1990; Ouzrout and Lerondelle, 1992). Few data are available about the phenotypic profile of immune cells in milk from healthy goats (Guiguen et al., 1996). The aim of this study was to evaluate the phenotypic composition of blood and milk leukocytes, and their activation status, in relation to CAEV infection.

2. Materials and methods

Blood and milk samples were obtained from two groups of 10 Alpine goats each, starting 60 days after kidding, over a period of 6 months. The first group comprised of healthy animals belonging to a herd free from CAEV infection, and the second group were CAEV infected animals belonging to a herd with a prevalence of more than 90% of infection. Some of the goats in the CAEV infected group showed clinical symptoms of arthritis. The goats were randomly selected among animals in their second or third lactation. The presence of CAEV infection was determined by ELISA (Pourquier, Montpellier France). CAEV was never observed in control animals during the study. A panel of seven monoclonal antibodies (mAbs) specific for surface leukocyte goat antigens was used (CD8, WC1, CD14, Serotec, Oxford, UK) [γδTcR, CD4, Class II MHC, B-B7 (CD21 like), VMRD Inc., Pullman, WA]. An indirect immunofluorescence technique for whole blood, previously described for phenotyping murine, bovine and feline leukocytes (Poli et al., 1996; Massi et al., 1998; Paltrinieri et al., 2003) was used. The analysis was performed on 10,000 cells using a FAC Sort flow cytometer (Becton Dickinson, San Jose, CA, USA), equipped with Cell Quest software. Blood lymphocytes were selected by using a back-gating technique on CD8 fluorescence (FS) versus side scatter (SSC) parameters (Byrne et al., 2000), to exclude monocytes from analysis. The percentage of positive lymphocytes for each surface marker was recorded. Monocytes (CD14 positive cells) were recorded as the percentage on total leukocytes. These values were then used to calculate the absolute number of cells bearing a specific marker by using total and differential white blood cell (WBC) counts. The data obtained from milk are reported as the percentage and absolute number of positive viable cells for each marker, because it was not possible, for most of the samples, to design the proper lymphocyte analysis gate on FS versus SSC dot plots (Guiguen et al., 1996). Data were analyzed by SPSS 14.0 (SPSS, Chicago, IL, USA), following a one-way ANOVA, with herd as a fixed factor and goat as a randomized factor.

3. Results

Significant differences ($P < 0.05$) in blood leukocyte subsets were observed between CAEV negative and positive goats, at different sampling time, mainly for CD8, γδTcR, and MHC class II bearing cells (Table 1). Total distribution of mean values showed significant effects ($P < 0.05$) for infectious status, sampling time and their interactions. The percentage of CD8 positive cells was greater in CAEV positive goats than in CAEV negative goats, with an increase in the difference between the two groups throughout lactation. At the last sampling, the number of CD8 positive lymphocytes in blood of CAEV infected goats was twofold greater than in CAEV negative goats. The same trend was observed for γδTcR bearing T cells in CAEV infected goats.

The mean value of the ratio for CD4/CD8, calculated for all of the samplings, was 2.14 and 1.47 for CAEV negative and CAEV positive goats, respectively. Differences between the ratios were significant ($P < 0.01$) throughout the entire lactating period, with the exception of the 2nd sampling.

Slight differences were observed for the WC1 positive subset. The CD4 and CD21 positive values were similar for both groups of CAEV positive and negative animals, except for the first sampling where both subsets were greater ($P < 0.05$) in CAEV negative animals. Expression of class II MHC molecules was greater for CAEV negative animals throughout the entire lactation. In both CAEV positive and negative groups, the number of CD14 positive leukocytes was similar throughout lactation, with the exception of the last sampling where there was a greater number of CD14 positive leukocytes for CAEV infected animals.

Significant differences ($P < 0.05$) in milk leukocyte subsets between CAEV negative and positive goats were observed for some of the sampling times for CD8, CD4, MHC class II and CD14 bearing cells (Table 1). Total distribution of mean values showed a significant ($P < 0.05$) effect for infectious status and sampling time with no significant interaction. Greater values were observed, in percentage, for CD8 and class II MHC positive cells in CAEV infected goats, while γδTcR T and CD14 positive cells were greater ($P < 0.05$) in healthy animals. Differences in milk leukocyte subsets (absolute number)