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Original article

Influence of chronic caprine arthritis-encephalitis virus infection on the population of peripheral blood leukocytes

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Abstract

The influence of caprine arthritis-encephalitis (CAE) virus infection on the population of peripheral blood leukocytes in goats was evaluated. For this purpose two groups of adult dairy female goats were formed. The experimental group consisted of 17 goats, which had been naturally infected for many years. The control group comprised 29 non-infected goats, which originated from CAE-free herd. All goats were clinically healthy. Whole blood was collected and tested in hematological analyzer and light microscope to assess the total number of leukocytes and the percentage of four leukocyte populations – neutrophils, eosinophils, monocytes and lymphocytes. Then, flow cytometry with monoclonal antibodies against several surface antigens (namely CD14, CD2, B-B2, CD4, CD8h, TCR-N6, WC1-N2 and WC1-N3) was performed to assess the proportion of lymphocyte subpopulations. Statistically significant differences ($\alpha \leq 0.01$) were observed only in the subpopulations of T lymphocytes – percentage of all subpopulations were significantly higher in the group of seropositive goats. No statistically significant differences were revealed with respect to the total number of blood leukocytes, the average percentage of blood leukocyte populations and proportions of both T and B lymphocytes.

Key words: caprine arthritis-encephalitis, flow cytometry, peripheral blood leukocytes, lymphocytes, goats

Introduction

Both caprine arthritis-encephalitis virus (CAEV) and maedi-visna virus (MVV) belong to the genus *Lentivirus*, family *Retroviridae*. For many years following their isolation in 1960 for MVV and 1980 for CAEV they had been recognized as distinct pathogens, infecting two different ruminant species – sheep and goats, respectively (Sigurdsson et al. 1960, Crawford et al. 1980). Last years have brought the new view of their nature, revealing that they are capable of crossing the interspecies barrier not only in experimental but also natural conditions (Leroux et al. 1997, Castro et al. 1999, Shah et al. 2004a, Pisoni et al. 2005). Nowadays both these viruses are classed together as small ruminant lentiviruses (SRLV), which are further split into five groups from A to E (Shah et al. 2004b, Grego et al. 2007).

Leukocytes are the main target cells for SRLV (Narayan et al. 1982). Monocytes, macrophages and dendritic cells are responsible for establishing life-long infection with SRLV (Narayan et al. 1982). Infected monocytes migrate to various tissues, mainly synovium, lung, udder and central nervous system, where they differentiate into macrophages (Haase 1986). Infected macrophages secrete inflammatory cytokines which attract lymphocytes and induce chronic immune-mediated inflammation of infected tissues (Zink et al. 1990) and may inhibit viral integration (Nimmanapalli et al. 2010). Only small percentage of infected goats develop clinical signs – chronic progressive arthritis, which results in lameness and eventually recumbency in older goats, indurative mastitis with hypogalactia, chronic interstitial pneumonia and wasting syndrome (Phelps and Smith 1993). Rarely, subacute leukoencephalomyelitis in kids can be observed (Cork et al. 1974, Kaba et al. 2011). Unlike most other members of the genus, such as human, simian or feline immunodeficiency virus, SRLV are unable to infect lymphocytes (Gorrel et al. 1992). This is probably why immunosuppression and opportunistic infections are no hallmark of lentiviral infections in small ruminants (Evermann 1990, Pétursson et al. 1991). However, SRLV infection exerts an influence on lymphocyte reactivity (Kaba et al. 2010).

There are very limited data on changes in the leukocyte phenotype profile during SRLV infection. They come from in-vitro models (Milhau et al. 2003) and only few small-scale clinical experiments (Wilkinson et al. 1995, Grezel et al. 1997, Jolly et al. 1997, Ponti et al. 2008).

The aim of the study was to characterize the influence of CAEV infection on the population of peripheral blood leukocytes.

Materials and Methods

Goats

Forty six female dairy goats were included in the study and split into two groups. All animals were 3-5 year-old, in full lactation, before mating and without any clinical signs of diseases other than CAE.

The experimental group consisted of 17 naturally infected goats, which originated from a herd where CAE seropositive animals have been diagnosed many times during a 10 year-long period preceding the study and where the disease has been confirmed by virus isolation (Kaba et al. 2009).

The control group comprised 29 goats from a herd free from CAEV infection. The clinical symptoms of a disease have never been seen in this herd and the status of animals has been confirmed by serological examination.

Infection with CAEV was confirmed or ruled out using serological method. Goats were tested with commercial immunoenzymatic test – ELISA (IDEXX CAEV/MVV Total Ab Screening Test). The test was performed according to the manufacturer's manual using ELISA reading device ICN Flow Titertek Multiscan Plus Mk11 (Labsystems, Espoo, Finland). CAE-positive goats were tested three times and CAE-negative twice, a year apart. All the tests performed in one goat had to give consistent results to classify a goat as infected or healthy.

Blood samples

Peripheral blood from the jugular vein was collected by venipuncture into 10 ml tubes containing 5 mM EDTA-2K (ethylenediaminetetraacetic acid-dipotassium salt).

Evaluation of blood leukocyte populations

Total number of leukocytes was determined in a HC 510 analyzer (Hycel, Rennes, France). Proportions of four different leukocyte populations – neutrophils, eosinophils, monocytes and lymphocytes – were determined by microscopic examination of whole blood smears in light microscope.

Evaluation of lymphocyte subpopulations

Flow cytometry (FACStrak, Becton-Dickinson, USA) and monoclonal antibodies (mAbs) were used to differentiate lymphocytes into T helpers,

Table 1. Monoclonal antibodies to goat lymphocyte antigens.

No	Monoclonal antibodies	Cell surface antigen	Isotype	Cell type specificity	References
1.	CAM36A	CD14 (M-M9)	IgG1	Monocytes	Davis et al. 1990
2.	MUC2A	CD2	IgG2a	T lymphocytes, NK cells	Davis and Hamilton 1993
3.	BAQ44A	B lymphocytes (B-B2)	IgM	B lymphocytes	Davis and Ellis 1991
4.	GC50A1	CD4	IgM	T helpers lymphocytes	Davis et al. 1987, Larsen et al. 1990
5.	CACT80C	CD8	IgG1	T cytotoxic, suppressor lymphocytes	Shafer-Weaver and Sordillo 1997 Lichtensteiger et al. 1993
6.	CACTB6A	TCR1-N6	IgM	$\gamma\delta$ T lymphocyte subpopulations	Mackay et al. 1989
7.	BAQ4A	WC1-N2	IgG1	CD4-CD8-TCR $\gamma\delta$ + T lymphocytes epitope N2	Davis et al. 1987
8.	CACTB31A	WC1-N3	IgG1	CD4-CD8-TCR $\gamma\delta$ + T lymphocytes epitope N3	Davis et al. 1990

Table 2. Total number of blood leukocytes and the average percentage of main blood leukocyte populations in the groups of seropositive (n = 17) and seronegative (n = 29) goats.

Leukocyte population	Leukocytes (G/L)		Eosinophils (%)		Neutrophils (%)		Monocytes (%)		Lymphocytes (%)	
	CAE+	CAE-	CAE+	CAE-	CAE+	CAE-	CAE+	CAE-	CAE+	CAE-
Serological status	CAE+	CAE-	CAE+	CAE-	CAE+	CAE-	CAE+	CAE-	CAE+	CAE-
Arithmetic mean	12.8	16.0	5.7	7.0	41.0	31.0	3.9	3.0	52.1	59.0
Standard deviation	2.8	4.3	3.9	3.8	15.2	10.6	3.0	2.3	14.0	12.2

* statistically significant differences ($\alpha \leq 0.01$)

Table 3. Average percentage of T and B lymphocytes in the groups of seropositive (n = 17) and seronegative (n = 29) goats.

Serological status	Lymphocytes T (CD2 ⁺) (%)		Lymphocytes B (B-B2) (%)	
	CAE+	CAE-	CAE+	CAE-
Arithmetic mean	62.7	53.0	39.8	26.0
Standard deviation	15.8	14.5	20.9	11.9

* statistically significant differences ($\alpha \leq 0.01$)

T cytotoxic/suppressor, $\gamma\delta$ T lymphocyte subpopulations, WC1-N2+ and WC1-N3+ cells and B lymphocytes (Table 1). Monoclonal antibodies used for immunophenotyping were purchased from VMRD Inc. (Pullman, USA) and the secondary antibodies were from Medac (Hamburg, Germany).

Fifty microliters of peripheral blood were incubated with 1 μ l mAb to immunophenotyping and 1 μ l secondary mAb (FITC-conjugated goat anti-mouse IgG+ IgM (H+L) to identify IgM isotype of mAb or PE- conjugated goat anti-mouse IgG, to identify IgG isotype of mAb) for 15 min at room temperature.

Erythrocytes were lysed with 1 ml FACS lysing solution (Becton-Dickinson, USA) for 10 min. Hemoglobin and erythrocyte cell debris were eliminated by washing in PBS (Sigma, USA). Blood leukocytes were suspended in 0.3 ml of PBS with 0.5% formaldehyde (Sigma, USA).

Statistical analysis

Significance of differences was evaluated using Student's t-test for independent variables. The par-

Table 4. Average percentage of particular subpopulations of T lymphocytes in the groups of seropositive (n = 17) and seronegative (n = 29) goats.

Serological status	Lymphocytes CD4 ⁺ (%)		Lymphocytes CD8 ⁺ (%)		Lymphocytes TCR1-N6 (%)		Lymphocytes WC1-N2 (%)		Lymphocytes WC1-N3 (%)	
	CAE+	CAE-	CAE+	CAE-	CAE+	CAE-	CAE+	CAE-	CAE+	CAE-
Arithmetic mean	52.8*	37.0*	38.3*	21.0*	34.9*	9.0*	38.7*	13.0*	31.3*	15.0*
Standard deviation	16.4	12.7	14.1	11.1	14.9	8.5	13.7	9.5	18.1	8.4

* statistically significant differences ($\alpha \leq 0.01$)

ametric test was chosen after the normal distribution of data and the equality of variances had been confirmed with Kolmogorov-Smirnov (K-S) test and Levene's test, respectively. Significance level of 0.01 ($\alpha \leq 0.01$) was assumed for t-Student's and 0.05 ($\alpha \leq 0.05$) for K-S and Levene's tests. All statistical calculations were performed with IBM SPSS Statistics 19.0.0 software.

Results

No statistically significant differences in the total number of leukocytes as well as in the average percentage of leukocyte populations were revealed between seropositive and seronegative goats (Table 2). Similarly, percentage of both T and B lymphocytes were comparable in both groups (Table 3).

Statistically significant differences were observed in the subpopulations of T lymphocytes – CD4⁺, CD8⁺, TCR1-N6, WC1-N2 and WC1-N3. Percentage of all these subpopulations of T lymphocytes were significantly higher in the group of seropositive than seronegative goats ($p < 0.01$) (Table 4).

Discussion

All clinical studies on the influence of SRLV infection on leukocyte phenotype profile conducted by now have included very limited number of animals (2-10 animals in each group) in various age (Wilkinson et al. 1995, Jolly et al. 1997, Ponti et al. 2008). Our study was performed on considerably larger groups (17 seropositive and 29 seronegative goats) of comparable animals (similar age and physiological status). As CAE is a chronic disease emphasis was laid on including goats, which had been naturally infected for sufficiently long time (all of them seroconverted at least 2 years before the study). Moreover, the significance level used for statistical testing has been set up on 0.01 ($\alpha \leq 0.01$). Those measures should increase credibility of obtained results.

Population of peripheral blood leukocytes differs considerably between healthy ruminants and healthy monogastric mammals. In goats, lymphocytes outnumber the other groups of leukocytes. In turn subpopulation of $\gamma\delta$ T cells, bearing receptor type 1 (TCR-1) and antigen WC1 (workshop cluster 1), accounts for approximately 70% of all lymphocytes. They are both memory and cytotoxic cells. Their proportion in peripheral blood tends to decline with age but at the same time they become more prevalent in mucosal membranes (Winnicka et al. 1999). Moreover, they occur in large percentage in the udder and milk (Ismail et al. 1996). The antigen WC1 is built of two epitopes, which may have different variants denoted as N1, N2, N3 and N4. The majority of WC1 antigens are composed of N1 and N2 epitopes although tiny populations of N1-N2+ as well as N1+N2- isoforms, containing either N3 or N4, exist (Davis et al. 1996).

Since monocytes are main target cells for SRLV, influence of the infection on the proportion of monocytes in peripheral blood was anticipated. Unlike in the studies of Kennedy-Stoskopf et al. (1989) and Jolly et al. (1997) as well as in the studies on HIV infection in humans (Smith et al. 1984) we could not show any statistically significant alteration in the proportion of monocytes as well as any other main subpopulations of leukocytes. Our results agree with the study of Wilkerson et al. (1995). The inconsistent results of the studies carried out to date suggest that there is no typical influence of SRLV infection on main populations of leukocytes, which can be regarded as a constant characteristic of CAE.

The only subpopulation affected by CAEV infection were T lymphocytes, both TCR1 and TCR2 positive. Interestingly, several studies reported slight decrease in CD4:CD8 ratio as a constant characteristic of the infection (Kennedy-Stoskopf et al. 1989, Werling et al. 1994, Jolly et al. 1997, Milhau et al. 2003). It was due to the drop of CD4⁺ lymphocytes more than because of CD8⁺ increase (Perry et al. 1995). The same tendency is typical for HIV infection in humans (Fauci et al. 1984). However, other studies showed

elevation of CD8⁺ lymphocytes with no concurrent change in CD4⁺ cells (Ponti et al. 2008). Such changes may be characteristic for early stage of the infection. Our study, performed on chronically infected animals, revealed statistically significant increase both in CD4⁺ and CD8⁺ lymphocytes. It may result from strong immunological stimulation due to long-lasting infection as all seropositive goats included in the study had been infected for more than two years. Given that the decrease of CD4⁺ lymphocytes is recognized as the main cause of life-threatening immunosuppression in the course of AIDS (Shnittman and Fauci 1994, Burger and Poles 2003), our results contribute to the statement that CAEV is rather non-immunosuppressing virus. The statement is substantiated by the results of Grezel et al. (1997) in which no relationship between serological status of goats and levels of T lymphocytes, both CD4⁺ and CD8⁺, could be revealed.

Statistically significant increase could be observed in $\gamma\delta$ T lymphocytes, regardless of the type of mAb used for their identification (TCR1-N6, WC1-N2, WC1-N3). The same results were obtained in other studies (Jolly et al. 1997, Ponti et al. 2008). Given that this sub-population of T lymphocytes predominates in the blood of ruminants (Winnicka et al. 1999) it seems to substantiate the statement that immune-mediated inflammation is the core of CAE pathogenesis.

Concluding, percentage of all subpopulations of T lymphocytes were significantly higher in the group of seropositive goats. No differences were revealed with respect to the total number of leukocytes, the average percentage of leukocyte populations and percentage of both T and B lymphocytes.

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