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Polymorphism of blood leukocyte acid phosphatase and the profile of peripheral blood lymphocytes in the first of lactation trimester of cows naturally-infected with bovine leukaemia virus

Abstract

This study covered a population of 91 cows aged 3-6 years. Enzootic bovine leukaemia (EBL) was diagnosed with ELISA and PCR tests. The assays were performed in the 1st, 2nd, and 3rd month after calving. Use was made of anti-bovine monoclonal antibodies (Mab), (VMDR Inc. Pullman-USA) and conjugates of FITC and R-PE (Medac – Germany) with an indirect immunofluorescence reaction (IMF).
A differentiation observed between the population numbers of CD19+ B lymphocytes and CD2+ T lymphocytes as well as the CD8+ T lymphocyte sub-population was found to be significant between cows with different genotypes of AcP. Moreover, a correlation was found between the polymorphism of AcP and EBL incidence in cows with reference to the number and percentage of CD19+ B lymphocytes (AcP polymorphism x EBL interaction). A significant differentiation in the profile of peripheral blood lymphocytes was observed between EBL-positive and EBL-negative cows as well as over the first three months after calving.
The results reported in this study seem to indicate the potential contribution of a genetic predisposition connected with the expression of biological functions of the blood leukocyte acid phosphatase system in the activation and proliferation of these cells.

Key Words: cattle, enzootic bovine leukaemia, lymphocyte CD markers, IMF, interaction

Zusammenfassung

Titel der Arbeit: **Polymorphismus der sauren Phosphatase der weißen Blutkörperchen und das Profil der Lymphozyten des peripheren Blutes im ersten Laktationstrimester bei Kühen, infiziert mit dem Virus der bovinen Leukämie**
Den Untersuchungen wurden 91 Kühe im Alter von 3 bis 6 Jahren unterzogen. Die enzootische bovine Leukose (EBL) wurde anhand des ELISA (Rhône-Poulenc – Frankreich) und des PCR Tests diagnostiziert. Die Bestimmungen wurden im 1., 2. und 3. Monat nach dem Kalben durchgeführt. Es wurden monoklonale Antikörper (VMDR Inc. Pullman - USA) sowie Konjugate aus FITC und R-PE (Medac - Deutschland) in einer mittelbaren Reaktion der Immunofluoreszenz (IMF) eingesetzt.
Es wurde eine wesentliche Differenzierung der Größe der B CD19+ Lymphozytenpopulation und den T CD2+ Zellen sowie der Subpopulation von T CD8+ Lymphozyten bei den Kühen nachgewiesen, die sich genetisch voneinander unterscheiden. Ferner wurde eine gegenseitige Abhängigkeit zwischen dem Polymorphismus der sauren Phosphotase (SP) der weißen Blutkörperchen und der Erkrankung von Kühen an enzootischer boviner Leukose in Bezug auf Anzahl und Prozentsatz der B CD19+ Lymphozyten (Interaktion Polymorphismus SP x EBL) festgestellt. Darüber hinaus konnte eine wesentliche Differenzierung des Populationsprofils und der Subpopulation von Lymphozyten des peripheren Blutes zwischen den EBL+ und EBL- Kühen sowie in dem Zeitraum der ersten drei Monate nach dem Kalben ermittelt werden. Die erzielten Ergebnisse weisen darauf hin, dass es möglich sein könnte, dass die genetische Veranlagung, die mit der Expression der biologischen Funktionen des polymorphen Systems der sauren Phosphatase der weißen Blutkörperchen verbunden ist, bei der Aktivierung und Proliferation dieser Zellen beteiligt ist.

Schlüsselwörter: Milchrind, enzootische bovine Leukose, IMF, Interaktionen, Lymphozyten-Unterpopulation

1. Introduction

Acid phosphatase (AcP) (EC 3.1.3.2) of blood leukocytes constitutes an important element of an enzymatic equipment of grain-like structures of lysosome cells of the blood leukocyte system. In Black-and-White cattle, this enzyme demonstrates a genetically-controlled polymorphism determined by a pair of autosomal alleles. The dominant gene AcP^B controls the synthesis of B isoenzyme present in AB phenotype. This phenotype was determined by two genotype groups: dominant homozygote B/B and heterozygote B/b. The recessive gene AcP^b in homozygous genotype determines phenotype A, in which isoenzyme B does not occur. Fraction A, which is encoded independently of a genetically-determined acid phosphatase polymorphism, occurs in both phenotype groups (KACZMARCZYK and WALAWSKI, 1992). The AcP polymorphism demonstrates associations with blood leukocyte morphological differentiation and granulocyte metabolic efficiency (KACZMARCZYK et al., 1989; KACZMARCZYK and TAUBE, 1990). These relationships seem to indicate the possible participation of AcP in natural defense mechanisms against pathogens (KACZMARCZYK et al., 1999). Bovine leukaemia virus (BLV) is a contagious agent responsible for the development of enzootic bovine leukaemia (EBL). The virus spreads easily among animals and its treatment often fails. Cases of new infections in areas previously recognised as leukaemia-free are occurring sometimes (RUŁKA et al. 2001). What is more, a lack of an effective vaccine disables prophylactic activities. Infected animals are usually characterised by lower milking results, poorer breeding performance and a shortened productive life span (POLLARI et al., 1992; DA et al., 1993; D'ANGELINO et al., 1998). Naturally-occurring susceptibility or resistance to BLV infection has been the subject of numerous studies carried out recently (LEWIN et al., 1999). A significant role in the organic defense against BLV has been ascribed to BoLA class II genes, mainly of locus DRB3.2.

Recognition of the genetic mechanisms able to protect an organism against EBL development as well as genetic markers of susceptibility/resistance to BLV infection would be of significant importance in fighting this disease. It would enable identification of reproductive bulls resistant to BLV infections as well as selection of individuals with genetically-determined resistance to this disease.

The aim of the study was to determine the relationship between a monogenic system of blood leukocyte acid phosphatase and the differentiation of the population sizes (cell number and percentage) of CD19+ B lymphocytes and CD2+ T cells as well as the differentiation of T - cytotoxic (CD8+) and T - helper lymphocytes (CD4+) subsets in the first months of lactation of cows naturally-infected with bovine leukaemia virus.

2. Material and methods

Studies were performed on the population of 91 Black-and-White breed cows, aged 3-6 years, from a leukaemia-dominated herd from a farm located in the province of Elbląg. The animals were reared indoors in stalls under good zoohygienic conditions. They were fed a balanced energy-protein diet. Enzootic bovine leukemia was diagnosed with the immuno-enzymatic ELISA kit (Rhone-Poulenc, France) and a PCR molecular test. Blood for analysis was sampled from the jugular vein using heparin as an anticoagulant. The assays were performed in the 1^{st}, 2^{nd}, and 3^{rd} month after calving. The cows who obtained at least one positive result in a serological or

morphological test were designated as EBL-positive (EBL+), while those who obtained a negative result in the three subsequent months of lactation - as EBL-negative (EBL-).

2.1. Determination of the polymorphism of blood leukocyte acid phosphatase

Polymorphism of blood leukocyte acid phosphatase was assayed electrophoretically as described previously (KACZMARCZYK, 1986). A 0.85% NH_4Cl solution was used for the lysis of erythrocytes and a 0.85% NaCl solution for rinsing of isolated blood leukocytes. Prior to electrophoresis, the blood leukocytes were thawed and frozen, and the obtained supernatant (15 min, 500xg, +4^{o}C), supplemented with bromophenol blue (indicator of protein migration), was transferred into the wells of 1% agarose gel. Electrophoretic buffer was a 0.1 M solution of sodium veronal, with pH 8.4 and ionic strength of 0.05, which after being diluted with distilled water (1:2) was used for gel preparation. The electrophoresis was run at a temperature of +4^{o}C and at current voltage of 200V, 250V and 300V changed threefold in 30-min intervals. Over the last 45 min of electrophoresis, the voltage was kept constant at 350V. Gels were incubated at a room temperature in a 50 mM sodium veronal–sodium acetate buffer, at pH 4.9-5.0, containing 0.1% sodium α-naphthyl phosphate, 0.1% Fast Red TR Salt, and 5 mM $MnCl_2$. Electrophoregrams were fixed and stored in a 5% solution of acetic acid.

2.2. DNA isolation

Genomic DNA was isolated from blood leucocytes obtained as a result of erythrocyte lysis with a 0.85% solution of NH_4Cl. To isolate DNA, a Wizard Genomic DNA Purification Kit was used following protocol of the manufacturer (Promega, USA). The amount and the quality of DNA isolated were assayed spectrophotometrically (GeneQuant, Pharmacia, USA) and electrophoretically in a 2% agarose gel. Visualization of electrophoregram enabled an evaluation of the genomic DNA isolated.

2.3. PCR protocol

A fragment of BLV genome with a length of 364 bp located in the area of “gag” 628-1806 bp gene and a fragment of kappa gene of milk casein with a length of 273 bp were amplified from genomic DNA (an indicator of a proper course of the PCR reaction). The earlier described PCR protocol (CZARNIK et al., 2000) was used with primers, synthetised by the MWG-Biotech company (Germany), and the following nucleotide sequence:
leu1: 5’GTCGACAACCTTCCCGACGG3’
leu2: 5’GACAGTCTCGTTTCCAATGG3’
kapp1: 5’GAAATCCCTACCATCAATACC3’
kapp2: 5’CCATCTACGCTAGTTTAGATG3’

2.4. Immunofluorescent-staining

Relative frequency of particular subsets of lymphocytes studied has been established by means of a routine single immunolabelling. Approximately $1.5x10^7$ of white blood cells was diluted in phosphate buffered saline (PBS), pH 7.4 depleted of Ca^{2+} and Mg^{2+} ions (Biomed, Lublin, PL), containing 1% bovine serum albumin (BSA) and

0,02% natrium azide and incubated with particular antiserum (described below) for 30 min at 4 °C. Following mouse monoclonal antibodies (mAb) (in a working dilution of 0.5 μg/50 μl each), raised against bovine antigens, have been used: anti-B-B2 that binds to a molecule similar to human CD19 (BAQ44A; UNGAR-WARON et al., 1997; WINNICKA et al., 1999), anti-BoCD2 (MUC2A; DAVIS et al., 1993), anti-BoCD4 (GC50A1; DAVIS et al., 1987), anti-BoCD8 (CACT80C; SHAFER-WEAVER and SORDILLO, 1997) and anti-WC1-N2 (BAQ4A; DAVIS et al., 1987) all purchased from VMDR Inc. Pullman, USA. Afterwards, the cell suspension was centrifuged (at 200 g), resulting pellet re-suspended and washed (5 min) and then centrifuged again. This procedure was repeated three times. After the last wash, the suspension was then incubated with FITC- or R-PE-conjugated goat anti-mouse IgG (Medac, FRG, working dilution of 0.1 μg/50 μl each) for 30 min at 4 °C, in order to visualize the primary antibody-antigen complex. After a final wash, a cellular smear has been prepared and viewed under a fluorescence microscope (Axiolab-Zeiss) equipped with an appropriate filter sets.
The control was prepared with the same protocol but without the addition of the primary antibody**.**

2.5. Counting of lymphocytes and statistical analysis of data

Counting was based on 200 cells registered in the visual field. Calculations were made to determine the absolute number (total number of leucocytes (WBC) x % of cells with a specified phenotype CD /100) and percentage of lymphocytes with a specified surface marker.
The results obtained were subjected to a statistical analysis (mean ± S.D.). In the analysis of the normal distribution parameters the values characterising some traits (cell number, T CD8+ lymphocyte percentage, T CD4/CD8 lymphocyte ratio) were subjected to logarithmic transformation ($\log_{10}$). The logarithmic values were used for further calculations. A three-factorial analysis of variance (General ANOVA/MANOVA) was applied for the factor sets. The analyses focused on the effect of the following factors: AcP polymorphism (phenotype A, phenotype AB), BLV infections (EBL+, EBL-) and month of lactation (1, 2, 3 months after calving), as well as the impact of interactions between these factors on the size (number, cell percentage) of blood lymphocyte population and sub-population. A comparative analysis of means between particular groups was performed with the POST HOC TEST method of Scheffe. Statistical analyses were done with STATISTICA 6.0 software.

3. Results

3.1. Characteristics of the experimental material

The ELISA and PCR test applied made it possible to identify 72 EBL-positive cows (79.1% of population) and 19 EBL-negative cows (20.9% of population). The AB phenotype was found in 72 cows (79.1%), while A phenotype in 19 cows (20.9%). The AB phenotypic group included 58 EBL-positive cows (80.6%) and 14 EBL-negative cows (19.4%), whereas the group with A phenotype included 14 EBL-positive cows (73.7%) and 5 EBL-negative cows (26.3%).

Table
Analysed factors and the profile of peripheral blood lymphocytes in the examined cows (Die analysierten Faktoren und das Profil der Lymphozyten des peripheren Blutes bei den untersuchten Kühen)

Cell surface marker	Statistical measures	Phenotypes of AcP (1)		Result of diagnostic test (2)		Months of lactation (3)			Interaction			
		A	AB	EBL+	EBL-	I	II	III	1x2	1x3	2x3	1x2x3
B-B2 (CD19+)	$\bar{x}$	7,63^{a}	4.97^{a}	6.04^{A}	3.54^{A}	5.26	5.83	5.45	*			
(10^9/L)^{X}	SD	6.45	2.76	4.24	1.43	3.51	3.77	4.59				
(%)	$\bar{x}$	49.95^{B}	42.62^{B}	45.53^{C}	38.84^{C}	41.43^{D}	46.10^{D}	44.90	*		**	
	SD	14.88	11.00	12.11	11.39	11.94	12.14	12.30				
BoCD2 (10^9/L)^{X}	$\bar{x}$	3.19^{b}	3.81^{b}	3.87^{E}	2.98^{E}	4.11	3.86	3.04				
	SD	1.69	1.88	1.94	1.25	2.31	1.66	1.27				
(%)	$\bar{x}$	26.05^{F}	34.89^{F}	32.92	33.55	35.67	34.19	29.06	P=0.07			
	SD	10.52	12.25	12.96	10.19	13.24	12.32	10.67				
BoCD4 (10^9/L)^{X}	$\bar{x}$	2.39	2.32	2.45^{G}	1.89^{G}	2.49	2.44	2.05				
	SD	1.56	1.35	1.45	1.04	1.75	1.14	1.18				
(%)	$\bar{x}$	18.37	20.72	19.99	21.15	20.56	21.17	18.86			*	
	SD	7.13	7.97	7.70	8.43	8.61	7.34	7.44				
BoCD8 (10^9/L)^{X}	$\bar{x}$	1.17^{c}	1.37^{c}	1.35	1.22	1.34	1.48	1.15				
	SD	0.69	0.72	0.73	0.66	0.80	0.77	0.52				
(%)^{X}	$\bar{x}$	10.18^{d}	12.88^{d}	11.90^{H}	13.91^{H}	12.22	13.40	11.24	P=0.06			
	SD	5.70	6.55	6.62	5.66	6.31	7.47	5.24				
CD4/CD8^{X}	$\bar{x}$	2.22	1.96	2.07^{e}	1.81^{e}	2.09	1.94	2.01			*	
	SD	1.20	1.17	1.20	1.09	1.18	1.03	1.34				

^{X} Statistical calculations were made on logarithmic values ($\log_{10}$)
Mean values denoted the same of small letter are statistically different at $P \leq 0.05$.
Mean values denoted the same of capital letter are statistically different at $P \leq 0.01$.
1 x 2: The interaction of AcP polymorphism and result of diagnostic test; 2 x 3: The interaction of result of diagnostic test and lactation months; * $P \leq 0.05$; ** $P \leq 0.01$

3.2. Lymphocyte population and sub-population profile in the experimental cows

An analysis of the AcP polymorphism effect on the population number of peripheral blood lymphocytes showed a significant differentiation of the number and percentage of B lymphocytes of CD19+ phenotype, T lymphocytes of CD2+ phenotype, and T cells of CD8+ phenotype (Table). The cows with A phenotype, were characterised by a significantly higher number of CD19+ B cells (7.63x10^9/L; 49.95%) ($P \leq 0.05$; $P \leq 0.01$ respectively), and significantly lower numbers of CD2+ (3.19x10^9/L; 26.05%) ($P \leq 0.05$; $P \leq 0.01$ respectively) and CD8+ T lymphocytes (1.17 x10^9/L; 10.18%)

(P≤0.05), compared to the cows with AB phenotype (4.97x10^9/L; 42.62% as well as 3.81x10^9/L; 34.89% and 1.37x10^9/L; 12.88%, respectively). Additionally, a negligibly higher value of CD4 to CD8 T lymphocyte ratio was reported in those cows, though the differences were not confirmed statistically (Table).

An analysis of the EBL effect on the differentiation of population and sub-population size of peripheral blood lymphocytes revealed a significantly higher number (P≤0.01) and percentage of CD19+ B lymphocytes as well as a significantly higher (P≤0.05) value of CD4/CD8 T lymphocyte ratio in EBL-positive cows, compared to EBL-negative animals (Table). In these cows, significantly (P≤0.01) higher numbers of CD2+ and CD4+ T lymphocytes as well as a significantly (P≤0.01) lower percentage of CD8+ T lymphocytes were reported compared with healthy animals. The AcP polymorphism x EBL interaction was found (P≤0.05) to affect the number and percentage of B lymphocytes with CD19+ phenotype (Fig. 1). It was manifested by the highest number of (9.16x10^9/L; 53.49%) CD19+ B lymphocytes reported in the peripheral blood of EBL-positive cows with A phenotype, and by their lowest number (3.58x10^9/L; 38.31%) noted in EBL-negative cows with AB phenotype. In the EBL-positive cows with A phenotype, the assays also showed a lower percentage of CD2+ (24.24%) and CD8+ (8.71%) T lymphocytes compared to healthy animals with AB phenotype (34.49% and 13.70%, respectively) (Fig. 2). However, the differences between those means were not confirmed statistically (P=0.075 and P=0.064).

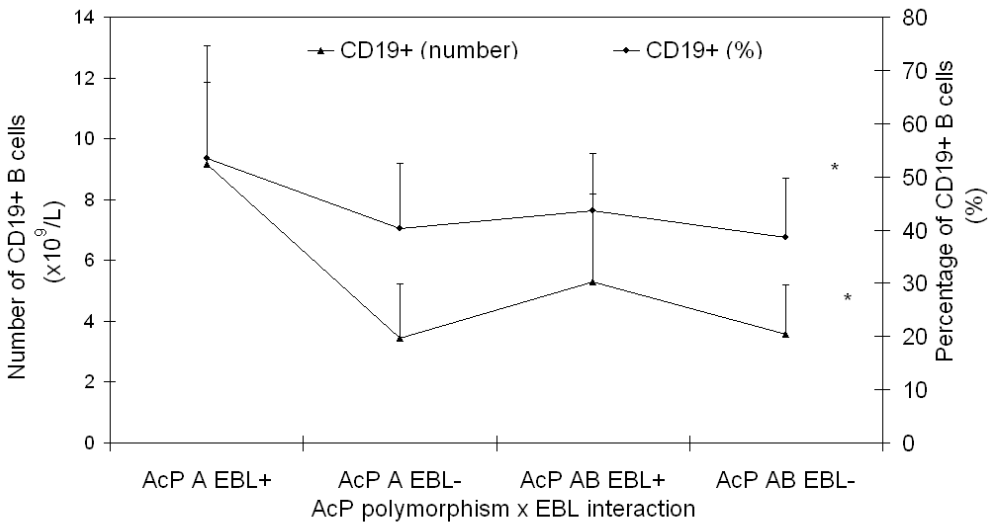

Fig. 1: The effect of AcP polymorphism and EBL interaction on the number and percentage of CD19+ B lymphocytes (Die Auswirkung der Interaktion zwischen AcP und EBL auf Anzahl und den Prozentsatz der B CD19+ Lymphozyten)

CD19+ (Number) - statistical calculations were made on logarithmic values (log_{10}); * P≤0.05

An analysis of the qualitative changes in the particular months of lactation showed the highest percentage (46.10%) of CD19+ B lymphocytes in the second and the lowest (41.43%) in the first month of lactation (P≤0.05) (Table). Similar changes were reported with respect to an absolute number of those cells, however the differences were not confirmed statistically. An opposite tendency was demonstrated by CD2+ T lymphocytes, the highest number of which (4.11x10^9/L, 35.67%) was reported in the

first month of lactation and the lowest one ($3.04x10^9$/L, 29.06%) in the third month of lactation (Table). The differences between the analysed months were not confirmed statistically (P=0.196 and P=0.082, respectively). In that period, no significant differences were reported in the value of the CD4/CD8 T lymphocyte ratio (Table).

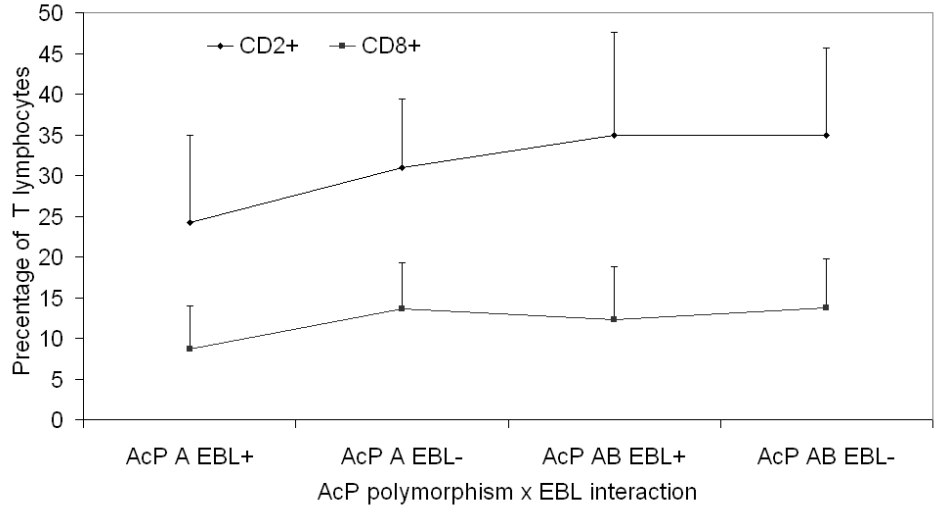

Fig. 2: The effect of AcP polymorphism and EBL interaction on the percentage of CD2+ and CD8+ T lymphocytes (Die Auswirkung der Interaktion zwischen AcP und EBL auf den Prozentsatz der T CD2+ Lymphozyten und CD8+ Lymphozyten)
CD8+ - statistical calculations were made on logarithmic values (log_{10}); The interaction non-significant (P=0.075 and P=0.064 respectively)

However, a significant (P≤0.01) interaction month of lactation x EBL was found to affect the percentage of CD19+ B lymphocytes (Fig. 3). In the BLV-positive cows, that percentage was alike over the whole lactation trimester (44.20%, 47.50%, and 44.80%), whereas in the EBL-negative cows – it was increasing in the subsequent months of lactation (30.90%, 40.62%, and 45.36%). The lactation month x EBL interaction was also observed to affect the percentage of CD4+ T lymphocytes (Fig. 3) as well as the ratio of CD4 to CD8 T lymphocytes (Fig. 4). That interaction was manifested by low values of those parameters reported in the EBL-negative cows in the first month after calving, their similar values in the second month after calving (irrespective of the state of health of the animals) as well as a significant (P≤0.05) differentiation of CD4+ T lymphocyte percentage between EBL-positive and EBL-negative cows in the third month after calving. The values of CD4 to CD8 T lymphocyte ratio showed an opposite tendency in the third month of lactation, however the differences reported between the EBL-positive and EBL-negative cows were not confirmed statistically.

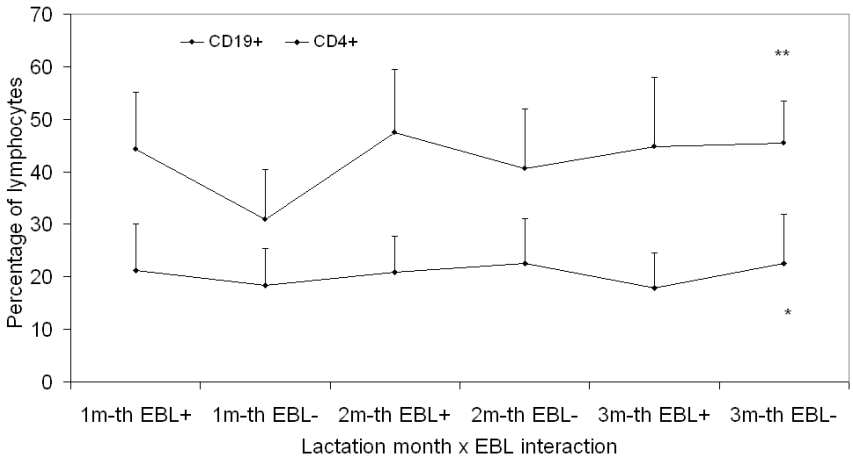

Fig. 3: The effect of lactation month and EBL interaction on the percentage of CD19+ B cells and CD4+ T lymphocytes (Die Auswirkung der Interaktion zwischen Laktationsmonat und EBL auf den Prozentsatz der B CD19+ Zellen und T CD4+ Lymphozyten)
* P≤0.05; ** P≤0.01

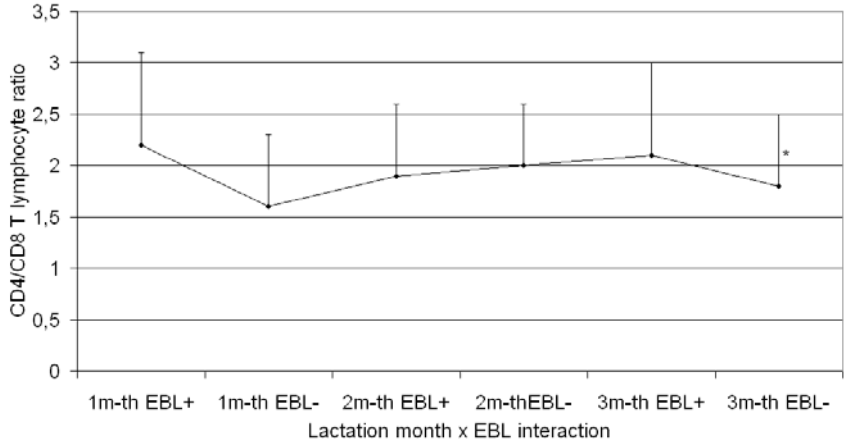

Fig. 4: The effect of lactation month and EBL interaction on CD4/CD8 T lymphocyte ratio (Die Auswirkung der Interaktion zwischen Laktationsmonat und EBL auf die Relation von T Lymphozyten und CD4/CD8 Lymphozyten)
Statistical calculations were made on logarithmic values (log_{10}); * P≤0.05

4. Discussion

In the case of cows with A phenotype, a significantly higher number of CD19+ B lymphocytes as well as lower numbers of CD2+ and CD8+ T lymphocytes were recorded, compared with the cows with AB phenotype (Table). Moreover, a co-operate was observed between AcP polymorphism and EBL incidence with respect to the number and percentage of CD19+ B lymphocytes (AcP polymorphism x EBL interaction) (Fig. 1). A significantly higher number of those lymphocytes was found in the EBL-positive cows with A phenotype than in the healthy animals with AB phenotype.

A CD19 molecule occurs on the surface of all B cells irrespective of the stage of their development. After transformation of B lymphocyte into a plasmatic cell, this molecule undergoes atrophy (TEDDER and ISAACS, 1989). Evoking a positive signal of B or T lymphocyte activation requires activation of co-stimulating molecules. Cytoplasmatic domenes of these molecules, which include CD19 on B lymphocytes (FUJIMOTO et al., 1999) and CD2 on T lymphocytes (DANIELIAN et al., 1992), take part in modulation of signal transmission at the early stage of cell activation. The cross-linking of an antigen through BCR or CD19 results in a rapid phosphorylation of tyrosine residues in the area of CD19 cytoplasmatic domene (CHALUPNY et al., 1993, DOODY et al., 1996) as well as in the activation of numerous kinases (e.g. Lyn, Fyn and Lck from the Src family), (FUJIMOTO et al., 2000; INABE and KUROSAKI, 2002) and other molecules, which participate in the signalling of B lymphocyte activation.

The protein tyrosine kinases (PTKs Src, Syk) co-operate with protein phosphatases in a reversible phosphorylation of many effectors participating in the signal transduction from a membrane receptor complex to the nucleus (HERMISTON et al., 2002). This process must be precisely controlled to maintain homeostasis of the lymphatic system. Disturbances in its course may lead to immunological deficits, autoimmunisation and/or neoplasm development (LI and DIXON, 2000, HERMISTON et al., 2002, PENDARIES et al., 2003). An important negative regulator of BCR signalling is SHP-1 phosphatase, however, a detailed mechanism of its activity has not been recognised yet. In the first stage of BCR signalling, the SHP-1 phosphatase is claimed to demonstrate the ability to dephosphorylate/deactivate Lyn kinase associated with CD19. Thus, it may affect many intracellular biochemical events evoked by BCR ligation and, in this context, is likely to represent a very significant mechanism whereby SHP-1 realizes its inhibitory effects on BCR signalling (SOMANI et al., 2001). The SHP-1 phosphatase regulates also TCR signalling (NEEL, 1997; WILLIAMS et al., 1999). It is suggested that the enzyme may bind directly with ZAP-70 kinase, which plays a major part in TCR signalling. A direct activity of SHP-1 on tyrosine kinases Lck and Fyn is also possible (NEEL, 1997). Probably, SHP-1 phosphatase is necessary to modulate of interleukin 2 (IL-2) signalling (LORENTZ et al., 1996). The recruitment of SHP-1 to an interleukin 2 receptor (IL-2R) complex is thought to constitute a mechanism regulating IL-2 induced responses (MIGONE et al., 1998) and a loss of SHP-1 activity may contribute to lymphocyte T-derived neoplasmatic transformation observed in adult T-cell leukaemia (SCHULTZ et al., 1997; MIGONE et al., 1998). Moreover, the SHP-1 phosphatase is claimed to exert a positive or negative impact in different signalling pathways (NEEL and TONKS, 1997). Still, details of those interactions have not been recognised. The TCR signalling is also regulated by some other phosphatases (e.g. CD45, SHP2 as well as LMPTP). The low molecular weight PTPase (LMPTP) belongs to a separate class of phosphatases which are widely distributed in vertebrates and inverterbrates (ZHANG et al., 1998). In mammals, a single gene has the capacity of encoding two isoenzymes of that phosphatase (CIRRI et al., 1996). They were formed as a result of an alternative mRNA splicing, and differences refer to amino acid residues 40-73 in the mature protein structure. This enzyme is identified with blood erythrocyte acid phosphatase occurring in all major human tissues (DISSING et al., 1991; WO et al., 1992; ZHANG et al., 1998). The physiological function of LMPTP has not been identified in details

yet. Thus, this enzyme is thought to participate in the dephosphorylation of ZAP-70 kinase, by preventing its inactivation and - similarly to CD45 phosphatase – has a positive function in TCR signalling (BOTTINI et al., 2002). The relationship observed in this study between polymorphism of blood leukocyte acid phosphatase and population and sub-population number of CD19+ B lymphocytes, CD2+ and CD8+ T lymphocytes seems to indicate the possible participation of AcP, registered in peripheral blood lymphocytes in two genetically-determined isoforms, as one of the protein phosphatases in activating signal transduction from BCR and TCR into the nucleus. An association with an incomplete expression of acid phosphatase enzymatic function reported in recessive homozygotes (heifers, bull calves, and cows) seems therefore probable (KACZMARCZYK et al., 1989, KACZMARCZYK and TAUBE, 1990, KACZMARCZYK et al., 1999). An enzyme deficit may impair the dephosphorylation of important elements (e.g. CD19 or protein kinase) which participate in signal transmission from a membrane receptor complex into the inner part of the cell. It may result in an insufficient suppression of cell-activating signalling, which in turn may lead to lowering of the B lymphocyte activation threshold in individuals with A phenotype. It may enhance uncontrolled proliferation of B lymphocytes upon their activation by BLV antigens, which seems to be manifested by the AcP polymorphism x EBL interaction observed in the study. What is more, in T lymphocytes, this enzyme seems to interact with other signalling effectors. Thus, it may exert an opposite effect than in B lymphocytes, by acting as a negative regulator of CD2+ and CD8+ T lymphocyte activation. The lower number of CD8+ T lymphocytes reported in recessive homozygotes (A phenotype) may indicate an inhibited proliferation of cytotoxic T lymphocytes, compared to individuals with AB phenotype. Additionally, it may point to the suppression of the immune defence of the organism, which may lower the efficiency of BLV-infected cells destroying. Under these conditions, the virus could have spread more easily in the organism and eventually lead to the development of enzootic bovine leukaemia. A lower percentage of CD2+ and CD8+ T lymphocytes observed in the peripheral blood of the examined BLV-positive cows with A phenotype, compared to BLV-negative animals with AB phenotype (Fig.2) seems to confirm that suggestion, however, the differences between those groups were not confirmed statistically ($P=0.075$ and $P=0.064$, respectively). The enzootic bovine leukaemia is characterised by an excessive proliferation of immature B lymphocytes and a decrease in lymphocyte T percentage (GATEI et al., 1989; STOTT et al., 1991; TIZARD, 1992; STONE et al., 1995; WU et al., 1999). However, healthy cows demonstrate an opposite tendency in lymphocyte percentage, namely: 10-34% of B lymphocytes, 45-60% of T lymphocytes, as well as 15-40% of CD4+ and 12-17% of CD8+ T lymphocytes. The population and sub-population numbers of peripheral blood lymphocytes determined in this study for EBL-negative cows (Table) approximate the values reported by other authors (GATEI et al., 1989; STOTT et al., 1991; TIZARD 1992). The differentiation of the number and percentage of CD19+ B lymphocyte between EBL-positive and EBL-negative cows was confirmed statistically ($P\leq0.01$). Moreover, in EBL-positive cows, a significantly lower ($P\leq0.05$) percentage of CD8+ T lymphocytes, and significantly higher ($P\leq0.05$) ratio of CD4 to CD8 T lymphocytes were observed (Table). These cows were also characterised by a negligibly lower percentage of CD2+ and CD4+ T lymphocytes, compared to EBL-negative cows. However, the differences were not confirmed

statistically. The EBL-positive cows demonstrated a significantly higher ($P \leq 0.01$) absolute number of CD2+ and CD4+ T lymphocytes (Table). Similar results were reported by other authors (STONE et al., 1995; ISAACSON et al., 1998).

A mechanism inducing B lymphocyte expansion in EBL-positive animals has not been identified yet. The expansion may result from an increased proliferation and/or an increased survival of B lymphocytes (STONE et al., 2000; DEBACQ et al., 2002). It is postulated that the expansion of those cells is stimulated by virus proteins released from EBL-infected B lymphocytes, which active those cell directly or through cytokines released from non-infected T lymphocytes (STONE et al., 1995; STONE et al., 2000). A significant role in the blastogenesis process is ascribed to IL-2, released mainly from Th1 lymphocytes (CD4+) activated by viral proteins (STONE et al., 1995; TRUEBLOOD et al., 1998). An increased IL-2 expression in EBL-infected cows probably contributes to the development and/or maintenance of persistent lymphocytosis. The results obtained in this study indicate a relationship between the CD8+ T lymphocyte percentage and EBL incidence in cows. A lower percentage of those cells may enhance the escape of EBL-infected cells from the BoLA immune system control. The cytotoxic T lymphocytes (BoCD8) are able to recognise viral, bacterial or neoplasmatic antigens presented by BoLA class I molecules, and destroy them by secreting cytotoxic substances. Those lymphocytes also release of cytokines, which activate other cells (e.g. macrophages or granulocytes) to produce cytotoxic substances (KIM et al., 1998). Upon a low percentage of CD8+ T lymphocytes, the virus can more easily spread in the organism. A lower percentage of those cells reported in the study could have resulted from the suppressive activity of virus proteins on the mechanisms activating these lymphocytes as well as from genetic predispositions manifested by T lymphocyte disfunction. A significantly higher number and percentage of CD19+ B lymphocytes and a significantly lower number and percentage of CD2+ T lymphocytes (including CD8+ T lymphocytes) observed in the examined cows with A phenotype, compared to those with AB phenotype, could point to the share of genetic predispositions associated with the expression of biological functions in the polymorphic system of blood leukocyte acid phosphatase in the activation and proliferation of those cells. However, the presented hypothesis requires confirmation by further studies.

The peripheral blood of the investigated animals was also characterised by a significant differentiation in the percentages of CD19+ B lymphocytes in the analysed months of lactation (Table). The differentiation in the profile of peripheral blood lymphocytes in the periparturition period was reported by other authors (NAGAHATA et al., 1992; VAN KAMPEN and MALLARD, 1997; KIMURA et al., 1999; MEIROM et al., 1999). In comparing the last weeks of pregnancy with the first hours and days after calving, at first the percentages of CD2+, CD4+ and CD8+ T lymphocytes decreased significantly to gradually return to the pre-calving values in the 2-3 week after calving (VAN KAMPEN and MALLARD, 1997; KIMURA et al., 1999). On the other hand, while analysing the percentages of CD4+ and CD8+ T lymphocytes in the subsequent months of lactation, MEIROM et al., (1999) observed a significant increase in CD4+ T cell percentage and a decrease in the CD8+ T cell percentage in the first trimester, compared to the other trimesters of lactation. The quantitative changes in CD19+ B lymphocytes reported by different authors are also not completely unequivocal. NAGAHATA et al., (1992) did not observe any

significant changes in the CD19+ B cells in cows 2 weeks after parturition. In particular months of lactation, other authors reported the lowest percentages of CD19+ B lymphocytes in the first, and the highest in the second, trimester of lactation (MEIROM et al., 1999). Other authors did not report any significant differences between values of that index while comparing the period of advanced lactation (from the middle to the end) with the third day after calving (SHAFER-WEAVER et al., 1999). The quantitative changes reported for the peripheral blood lymphocytes in the first month after calving are often accompanied by suppressed immune functions of those cells (KIMURA et al., 1999) and an increased organism susceptibility to pathogenic infections (VAN KAMPEN and MALLARD, 1997).

The differentiation in the population and sub-population numbers of peripheral blood lymphocytes reported in this study and by other authors in cows just after calving is probably caused by physiological changes in the hormonal profile, resulting mainly from parturition and beginning of lactation (ROTH et al., 1982; SHAFER-WEAVER et al., 1999). In each organism, there were interactions between the nervous, hormonal and immune systems. As a result of those interactions, the immune system cells become target cells for numerous hormones and neuropeptides, and the substances produced by the immune system cells (cytokines, thymus hormones) affect the structure and functioning of the nervous system and endocrine glands (HARBOUR 1991, HOMO-DELARCHE and DARDENNE, 1991). These interactions may result in substantial quantitative changes in the cells participating in the immune mechanisms of cows in the periparturition period. In the presented research, interactions between leukaemia and lactation month were reported with respect to some populations and sub-populations of peripheral blood lymphocytes. They were manifested in a significant differentiation of CD19+ B lymphocyte percentage (Fig. 3) and CD4/CD8 T lymphocyte ratio (Fig. 4) in EBL-positive and EBL-negative cows in the first month after calving (I. month of lactation). Over that period, the EBL-negative cows were characterised by a significantly lower percentage of CD19+ B lymphocytes and a significantly lower ratio of CD4 to CD8 T lymphocytes, compared to the EBL-positive cows. Whereas in the two subsequent months of lactation, that differentiation was lower and not regular. An opposite tendency was reported for the percentage of CD4+ T lymphocytes (Fig. 3), since the interaction between the two factors was relatively low in the first two months after calving, to become high no sooner than in the third month after calving, when it was manifested by a statistically significant differentiation in the percentages of those cells between EBL-positive and EBL-negative cows.

The obtained results suggest that the blood leucocyte acid phosphatase polymorphism significantly differentiated the profile of the peripheral blood lymphocytes in the examined cows. More B CD19+ cells and less T CD2+ and CD8+ cells were found in cows with phenotype A. Additionally, a significant differentiation in the number of these lymphocytes between the leukaemic and the clinically healthy cows as well as a co-operation of acid phosphatase polymorphism with BLV infection (AcP polymorphism x leukaemia interaction) in their effect on the B CD19+ lymphocyte population size were found. This interaction was exhibited by a significantly larger number of B CD19+ cells in the EBL-positive cows with phenotype A than in the EBL-negative animals with phenotype AB. The significant differentiation in the percentage of B CD19+ lymphocytes in the first two months after calving and the

interaction of BLV infection and a month of lactation in their effect on the percentage of these cells were registered.

The relationship were reported for the first time indicate the possibility of an effect of genetic predispositions related to expression of biological functions of blood leucocyte acid phosphatase on the activation and proliferation of lymphocytes require confirmation in further studies on more material collected from other herds. The obtained results encourage further studies into the role of blood leucocyte acid phosphatase in the immunological processes in cattle.

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