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Leukocyte acid phosphatase and selected haematological indices in BLV infected cows

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Abstract. Acid phosphatase of blood leukocytes (AcP) is a lysosomal enzyme which occurs in granulocytes and lymphocytes, but is not found in monocytes. In cattle, the occurrence of AcP polymorphism appeared in the form of A and AB phenotypes controlled by two autosomal alleles. A statistically highly significant repeatability was observed for AcP activity measured in lymphocytes from cows resistant to BLV (bovine leukaemia virus) infection. The highly inherited AcP activity and monogenic nature of AcP polymorphism in cattle allowed us to find out an association between AcP polymorphism and activity of AcP as well as haematological indices. In this study, 60 cows reared in one herd were analysed. The blood samples were collected in the last month before calving and in the first week after calving. The results obtained from cows with phenotype A revealed a statistically higher activity of AcP in lymphocytes whereas a lower activity of this enzyme was recorded in granulocytes. Furthermore, statistically significant differences were also observed in leukocyte number, percentage of lymphocytes and percentage of neutrophils.

Key words: AcP activity, cows, enzootic bovine leukaemia (EBL), haematological indices, leukocyte acid phosphatase, polymorphism.

Introduction

Leukocyte acid phosphatase (monophosphate hydrolase) is lysosomal enzyme whose immunological protective function is taken into consideration. Acid phosphatase (AcP) in cattle shows a genetically controlled polymorphism determined by a pair of autosomal alleles. The dominant gene is expressed by the occurrence of AcP B variant and constituting the two-band AB phenotype. This phenotype was determined by two genotype groups: dominant homozygote (B/B)

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and heterozygote (B/b). The recessive gene is responsible for the lack of B variant recognized as the one-band A phenotype. The fraction A was recognized as a product coded irrespective of the genetic determination of AcP polymorphism (KACZMARCZYK, WALAWSKI 1992).

AcP enzymatic activity in the recessive A phenotype is much lower than in to the dominant AB phenotype individuals (KACZMARCZYK, TAUBE 1990). AcP polymorphism as well as enzyme activity are associated with leukocyte morphological differentiation and granulocyte metabolic efficiency (KACZMARCZYK et al. 1989, KACZMARCZYK, WALAWSKI 1990). In healthy cows, AcP activity in granulocytes and lymphocytes are characterised by a very high repeatability in successive physiological stages (WALAWSKI et al. 1993). This seems to confirm AcP polymorphism as a possible genetic factor differentiating natural resistance in cattle.

Viral diseases, including enzootic bovine leukaemia (EBL), are particularly significant in the cattle breeding population. The bovine leukaemia virus (BLV) is a member of the oncogenic retrovirus family. BLV infection takes place when the antigens localised in the external virion surface fuse with the specific B lymphocyte host cell receptor. The gene responsible for receptor synthesis has been recognised, but the mechanism of viral infection is still unknown except in some cases in selected cells of the leukocyte system (POPESCU et al. 1995). Existence of genetic determination of resistance/susceptibility to BLV infection was suggested. An important role was ascribed to BoLA complex class II genes (XU et al. 1993, LEWIN 1994, MIRSKY et al. 1998).

The effect of adsorption of the virus at lymphocyte cell surface can be different. Antigen isolation inside the phagocyte vacuole as well as fusion with lysosomes and digestion by lysosome hydrolases in the final stage of phagocytosis are possible. Quite often, however, RNA of the BLV genome undergoes reverse transcription and integrates with DNA of host cells in the form of a BLV provirus construction. BLV transformed lymphocyte cells are changed in terms of genetically controlled immunological and biochemical properties. This may lead to oncogene derepression and polyclonal expansion of the infected B cell population. This process is accompanied by significant bioenergetic and biochemical abnormalities.

The role of acid phosphatase as well as of other lymphocyte enzymes in BLV gene expression has not been explained yet. The aim of the present report was to determine the relationship between AcP leukocyte polymorphism, AcP activity and leukogram composition in cows with subclinical EBL.

Material and methods

Sixty cows, 4-6 years old, from a BLV-infected Polish Black-and-White herd were studied. Blood samples were collected from the jugular vein into heparinised

tubes. AcP polymorphism determinations and enzootic bovine leukosis (EBL) diagnosis were carried out a month before calving. The leukocyte count and leukogram as well as AcP activity in morphologically differentiated cells was performed 7 days after calving.

Analyses of polymorphism and leukocyte acid phosphatase activity were performed as described previously (KACZMARCZYK 1986, KACZMARCZYK, WALAWSKI 1986). EBL diagnosis was confirmed using two methods: the Agar Gel Immunodiffusion test (AGID) (MILLER, van der MAATEN 1976) and the PCR procedure. Genomic DNA was isolated from the blood by the Wizard Genomic DNA Purification System Kit (Promega). The BLV genome fragment of "gag" gene was amplified. Based on the data published by SAGATA et al. (1985), the following PCR primers were used:

BLV 1: 5' GCTGACAACCTTCCCGACGG3'
BLV 2: 5' GACAGTCTCGTTTCCAATGG3'

The PCR reaction was as follows: 2.5 μL 10 × PCR buffer (20 mM $MgCl_2$), and 1.0 μL of primer BLV1 and 1.5 μL of primer BLV2 (150 ng/μL each) (MGW Biotech), 1.5 μL dNTP-mix (2 mM each), 0.125 u Taq polymerase (Promega), about 1000 ng of DNA and template fresh millipore water to a volume of 25 μL. PCR programme: predenaturation for 2 min at 94°C followed by 35 cycles of: 30 sec 94°C, 60 sec 61°C, 45 sec 72°C and finished by 5 min at 72°C. PCR products as well as BLV-positive controls, negative control (no DNA) in the presence of PhiXi marker 174/Hae III were analysed by electrophoresis on a 1.5% agarose gel in TAE buffer. Electrophoresis was carried out in the following conditions: 80 V, 500 mA, 40 W for 30 minutes. PCR products were observed and photographed using a GDS7500 System (UVP).

The leukocyte counts were performed by standard methods. The total number of leukocytes per microlitre of blood was measured using a Picoscalle apparatus (MEDICOR – Budapest). White cell composition (leukogram) was analysed in MGG stained blood smears.

The general linear models procedure was used to determine the significance of the AcP activity effect.

Results and discussion

Diagnosis of enzootic bovine leukosis made it possible to detect 41 EBL-positive and 19 EBL-negative cows. In the group of EBL-positive cows AcP A phenotype was found in 6 cows and AcP AB phenotype in 35 cows (Figure 1). The variation of age was similar in the two phenotype groups. Cows with phenotype A originated from 6 bulls, and cows with phenotype AB from 22 bulls.

Results of leukocyte count and analysis of white cell composition are presented in Table 1. Most of the analysed indices are within the physiological norm,

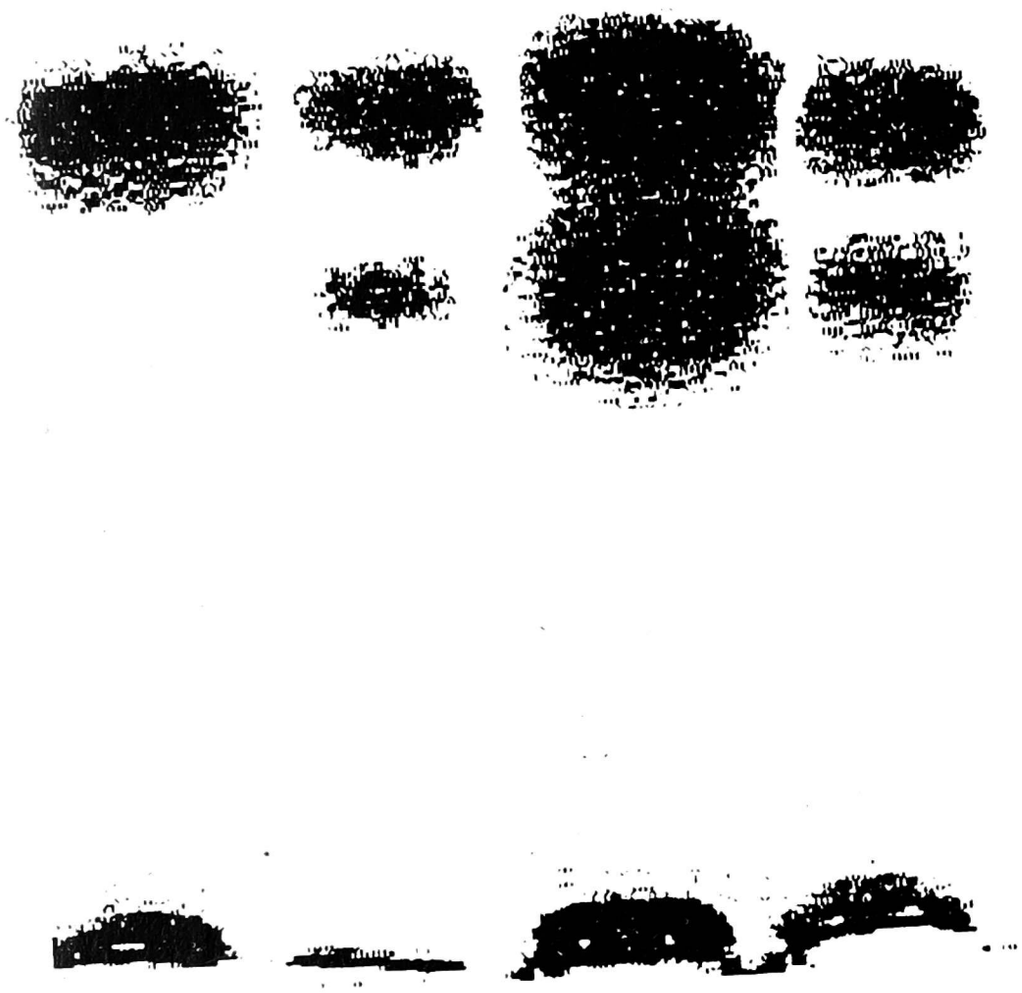

Figure 1. Acid phosphatase blood leukocyte phenotypes in cattle: from the left A, AB, AB, AB

Table 1. Characteristics of studied animals in view of analysed indices

Indices	$\bar{x}$	SD	Physiological standard (RICHTER et al. 1979)
Leukocytes (10^9/l)	14.64	4.86	5.0 - 10.0
Lymphocytes (1/1)	68.37	1.47	45.0 – 65.0
Neutrophils (1/1)	25.68	16.71	27.0 – 56.0
Eosinophils (1/1)	5.66	4.22	4.0 – 10.0
Basophils (1/1)	0.27	0.50	0.0 – 0.0
Monocytes (1/1)	0.29	0.45	2.0 – 9.0
AcP activity (total score) in:			
– total granulocytes	36.44	19.05	
– mature granulocytes	9.68	8.35	
– immature granulocytes	26.76	16.50	No data avaiable
– lymphocytes	38.80	11.26	
– monocytes	0.0	0.0	

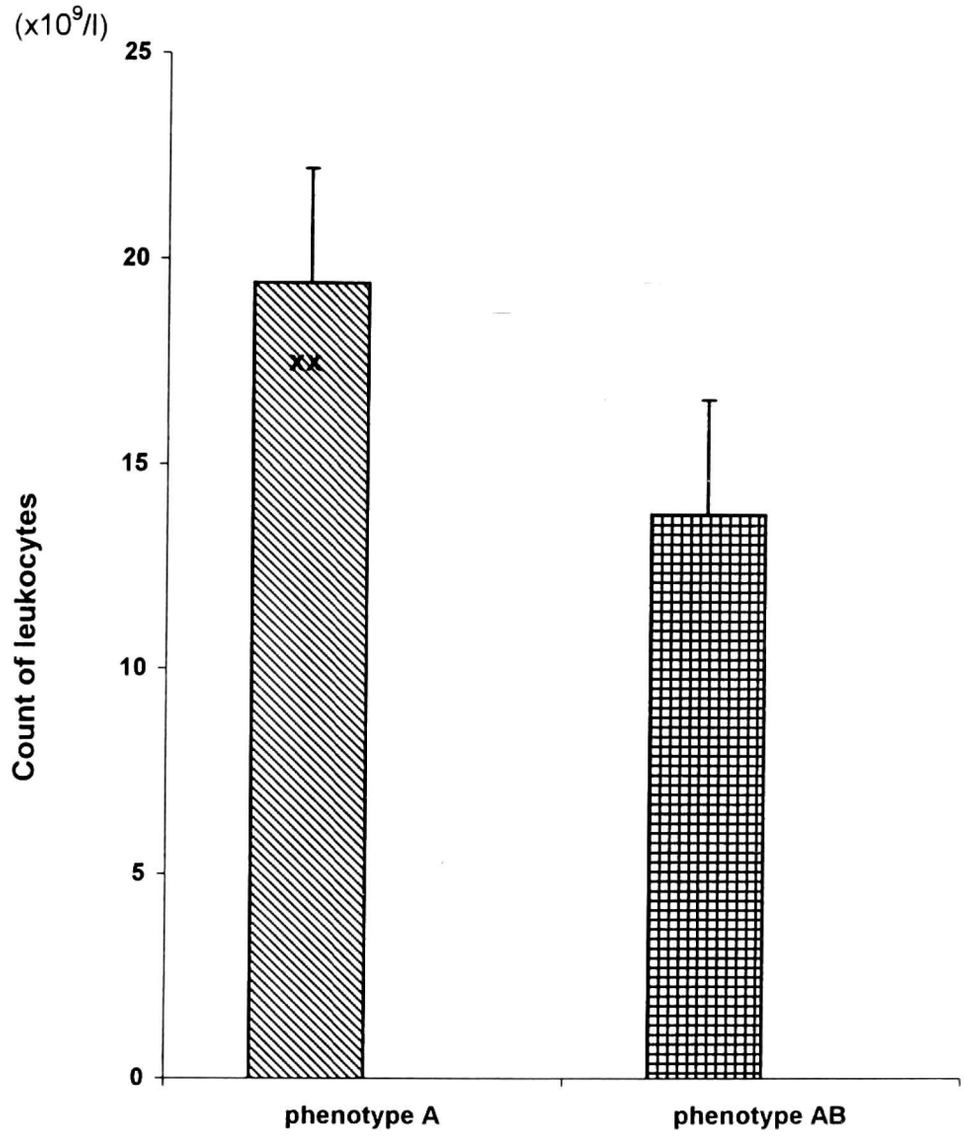

Figure 2. Count of leukocytes in cows with differentiated phenotypes of acid phosphatase

Legend: I – standard deviation (SD), ×× – difference statistically significant at P = 0.01

and only leukocyte count and lymphocyte percentage are slightly above the upper limit of the physiological standard (RICHTER et al. 1979). Moreover, activity of acid phosphatase in granulocytes (total score 36.44) and in lymphocytes (total score 38.80) confirmed results of earlier studies (KACZMARCZYK 1995). A higher enzyme activity was observed in immature granulocytes than in mature granulocytes, while no enzyme activity was found in monocytes (Table 1).

Analysis of the relationship between AcP blood leukocyte polymorphism and the level of registered haematological indices (Figure 2) showed a statistically significant difference in the leukocyte count, higher in AcP A individuals than in AcP AB phenotype ones.

The value of this index in recessive homozygotes (phenotype A) was higher than the upper populational normal range by around 100%.

Additionally, in cows with A phenotype, the percentage of lymphocytes was significantly higher, while the percentage of neutrophils and eosinophils was lower (Table 2).

Table 2. Leukogram in cows with differentiated phenotypes of leukocyte acid phosphatase

Indices (1/1)	Phenotypes of acid phosphatase			
	Phenotype A		Phenotype AB	
	$\bar{x}$	SD	$\bar{x}$	SD
Lymphocytes	83.83×	12.42	65.71	17.20
Neutrophils	13.33×	11.40	27.80	16.92
Eosinophils	2.33×	1.73	6.23	4.34
Basophils	0.23	0.24	0.50	0.43
Monocytes	0.0	0.0	0.31	0.47

× – difference statistically significant at P = 0.05

Cows with phenotype A showed a significantly higher AcP activity in lymphocytes and a lower activity in granulocytes (Figure 3). Substantial differences in enzyme activity were also noted in immature and mature granulocytes, but they were not statistically confirmed.

Changes in acid phosphatase activity registered in granulocytes and lymphocytes may be caused by carcinogenic cell transformation which influences the speed of metabolic changes taking place in leukaemic lymphocytes. The results of the previous research showed that EBL-positive cows showed a higher AcP activity in lymphocytes and lower in granulocytes as compared to cows free of BLV infection (KACZMARCZYK 1995). A increased enzymatic activity manifested in higher (by up to 67-89%) AcP positive lymphocyte counts was registered in sheep immunised with BLV virus (GRUNDBOECK, SZCZOTKA 1993) and in EBL-positive cows (by up to 83%) (RAICH et al. 1992). Changes of the enzyme reaction character from granular (enzyme–positive lysosomes) to diffusional (enzyme reaction in cytoplasm) were also observed, pointing to the existence of cells that are defective (SITARSKA et al. 1981).

In EBL-negative cows, statistically significant and stable values of repeatability coefficient of leukocyte AcP activity were found in three subsequent lactation periods (WALAWSKI et al. 1993). The coefficient of repeatability is defined as the ratio between the genetic variation and systematically appearing non-genetic factors and the whole phenotype variation, whereas the calculations made separately for the group of healthy cows not showing chronic disease symptoms illustrate only the isolated effect of additive genetic determination. Chronic diseases (mastitis, leukaemia) may change the genetic reliability of the estimated repeatability. Absolute values and relations of the regression coefficients calculated for

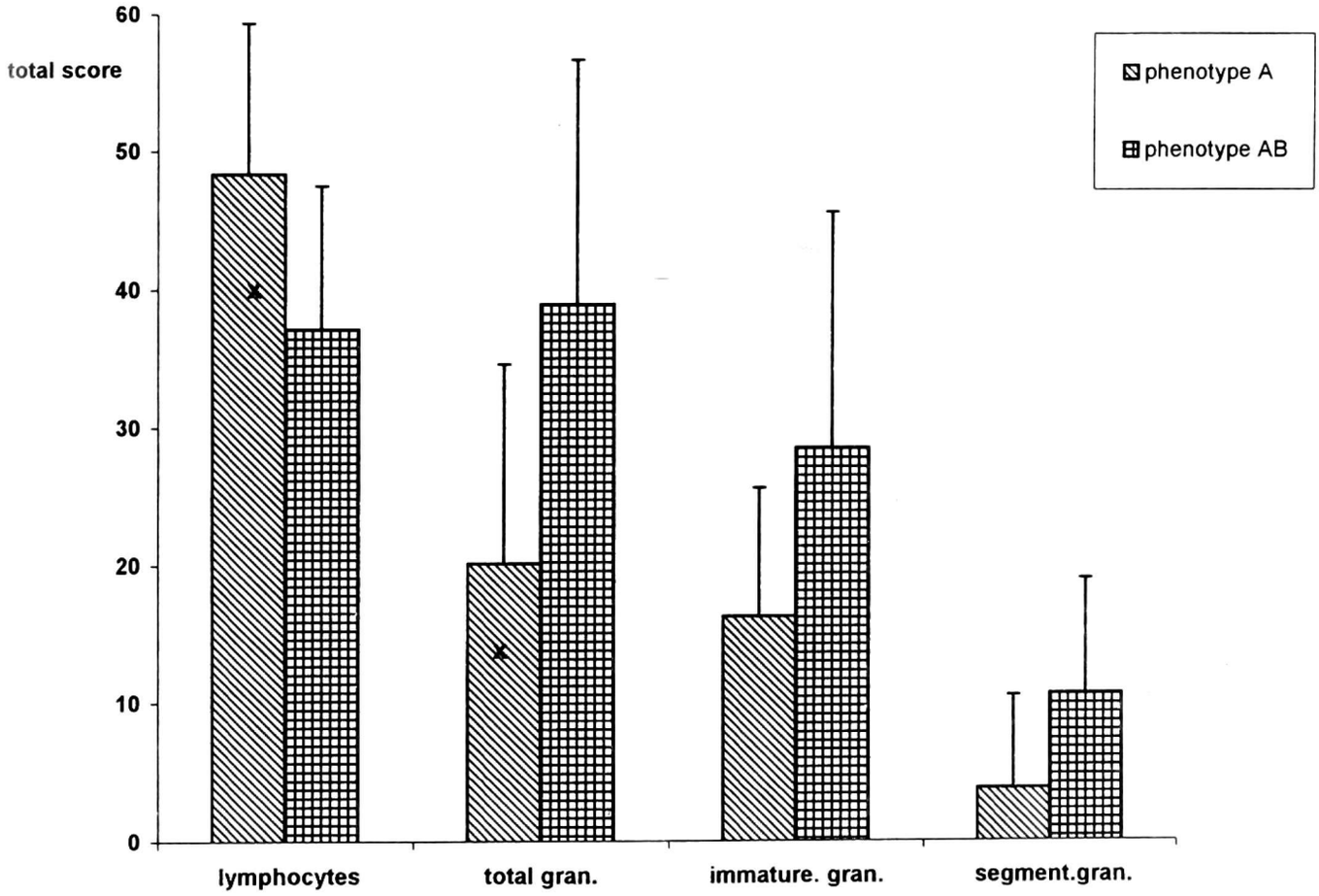

Figure 3. AcP activity in leukocytes of cows with differentiated phenotypes of acid phosphatase

Legend: I – standard deviation (SD), × – difference statistically significant at P = 0.05

particular features of animals in this group determine their selection suitability as heritability estimators. A statistically significant repeatability of AcP was recorded in lymphocytes from EBL-negative cows; this effect was stabilized in individual stages of the three subsequent lactations. These values calculated for AcP activity often exceed $b_{yx} = 0.5$ and indicate a high degree of genetic determination of this feature in the animals resistant to BVL infection (WALAWSKI et al. 1993).

The resistance and susceptibility to BLV infections was probably associated with the presence of a definite structural motif in the complex BoLA-DRB3 gene. It is expected that this gene, or an allele closely linked with this gene, may play a direct role in the counts of B BLV-positive cells (MIRSKY et al. 1998).

The main role in the destruction of cancer cells is ascribed to T lymphocytes, NK cells, macrophages and monocytes. Activation of T lymphocytes of CD4+ and CD8+ phenotypes by BVL virus antigens suggests a possibility that these cells may produce immunological mediators as well as their cytotoxic function expression (GATEI et al. 1993, MAGER et al. 1994). Appearance of acid phosphatase in granulosomes (a unique group of lysosomes observed only in cytotoxic lymphocytes) most probably plays a significant role in this process.

The results presented here are of a preliminary character only. The research will be continued on more numerous samples and on a population isolated B and T cells.

Conclusions

Most of the analysed indices in the examined leukaemia cows are within the physiological norm, except for the leukocyte count and the percentage of lymphocytes which slightly exceeded the upper limits. A significant relationship was observed between the polymorphism of acid phosphatase and the level of some indices. Cows with phenotype A had higher values of leukocyte count and lymphocyte percentage. Also lower neutrophil and eosinophil percentages, a lower acid phosphatase activity in granulocytes and a higher activity of this enzyme in lymphocytes were observed in this group.

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