POLYMORPHISM OF BLOOD LEUKOCYTE ACID PHOSPHATASE AND THE PROFILE OF BLOOD PLASMA PROTEINS IN COWS NATURALLY INFECTED WITH BOVINE LEUKAEMIA VIRUS

EWA KACZMARCZYK AND BARBARA BOJAROJC-NOSOWICZ

Department of Animal Genetics, Faculty of Animal Bioengineering, University of Warmia and Mazury, 10-719 Olsztyn, Poland
e-mail: ewagen@uwm.edu.pl

Received for publication December 29, 2005.

Abstract

The aim of the study was to determine the relationship between the blood leukocyte acid phosphatase polymorphism and BLV infection and blood plasma protein fraction composition in cows in the first trimester after calving. The studies were performed on the population of 64 Black-and-White breed cows, aged 3-6 years, from a leukosis-dominated herd. It was found that the blood leukocyte acid phosphatase polymorphism and the BLV infection significantly diversify the levels of \( \beta_1 \)- and \( \beta_2 \)-globulins and \( \gamma \)-globulin. Considering the essential immunological functions of the proteins constituting these fractions, the recorded relationships indicate different immunological conditions of the specimens carrying different AcP phenotypes and their different health states. Moreover, the BLV seems to modify the effect of the blood leukocyte acid phosphatase genotype on the \( \beta_2 \)- and \( \gamma \)-globulin fractions. The obtained results encourage further, more detailed studies into the individual proteins of the \( \beta_1 \)- and \( \beta_2 \)-globulin and \( \gamma \)-globulin fractions in the blood plasma of animals of different phenotypes of acid phosphatase and at different stages of enzootic bovine leukosis development.

Key words: cows, bovine leukaemia virus, blood proteins, leukocytes, polymorphism, acid phosphatase.

Blood leukocyte acid phosphatase polymorphism (AcP) in Black-and-White breed cattle is manifested by phenotypes A and AB which, in turn, are determined by a pair of autosomal alleles (14). The dominant gene is responsible for the synthesis of B isoenzyme occurring in the AB phenotype. This phenotype is determined by the dominant homozygote and heterozygote. The recessive gene in the homozygote is manifested by the lack of B isoenzyme and in individuals with the A phenotype (14). Fraction A of the enzyme, recorded in both phenotypes, is not determined by this gene pair. Blood leukocyte AcP polymorphism exhibits a relationship with the levels of some of the haematological and immunological indices (9, 10, 11, 13). These correlations indicate the possibility of AcP participation in the organism immunity processes. Some environmental factors may modify the natural relations between the animal genotype and processes occurring in the cell and favour pathogenic infections. Bovine leukaemia virus (BLV) is an exogenic, transactivating retrovirus responsible for the development of enzootic bovine leukosis (EBL) - a chronic neoplastic disease of the lymphatic system. The molecular and biological properties of this virus indicate its close relationship with the human T-HTLV-I and HTLV-II lymphocyte leukaemia virus (human T-cell leukaemia virus) and monkey T-cell leukaemia virus. BLV infection disregulates the host immune system at both humoral and cellular levels (15, 21) where the main role is played by the cytokines. They are likely to cause different patterns of disease progression (23). In addition, cytokines affect the scope, character and duration of the immunological response by influencing the cell activation, proliferation and differentiation (2, 8). As the result, they affect the concentration of acute-phase proteins which play different biological functions and co-operate both in the initiation and regulation of inflammatory response evoked by infection, neoplastic disease or other undesirable factors (5, 8). The acute-phase proteins occur in blood plasma and are mainly synthesized by hepatocytes and plasmocytes or originate from cells destroyed in the course of the disease. In pathological status, the levels of some proteins change and this is reflected in the qualitative and quantitative composition of blood plasma proteins (3, 24).

The results obtained to date indicating the possible participation of acid phosphatase in the organism defence mechanisms as well as the relationship between the BLV infections in cows and the differentiation in the blood plasma protein composition, which are poorly documented in the world-wide references, encouraged the authors to determine the relationship between the blood leukocyte acid phosphatase polymorphism and the
BLV infection and blood plasma protein fraction composition in cows in the first trimester after calving.

Material and Methods

**Animals.** The studies were performed on the population of 64 Black-and-White breed cows, aged 3-6 years, from a leukosis-dominated herd. The animals were reared indoors in stalls under good zoohygienic conditions. They were fed a balanced energy-protein diet. The herd was free from tuberculosis and brucellosis. Initial analyses were performed in the second half of the first month after calving and were continued in the second and third month of lactation in 4-week intervals.

**Diagnosis of enzootic bovine leukemia.** Diagnoses of enzootic bovine leukemia were confirmed using the ELISA (Rhône-Poulenc, France) and polymerase chain reaction (PCR). The cows which were positive in the ELISA or PCR test were designated as EBL+, while those which were negative in 3 subsequent months of lactation were designated as EBL-.

To isolate genomic DNA, a reagent-grade Wizard Genomic DNA Purification Kit and isolation procedure provided by the producer (Promega USA) were used. A fragment of a virus genome with a length of 364 bp located in the area of gag 628-1806 bp gene and a fragment of milk kappa casein (CASK) gene with a length of 273 bp were amplified from genomic DNA (an indicator of a proper course of PCR reaction). The previously described primers (TIB-MOLBIOL-Poland) and the PCR protocol were applied (9). The ELISA was carried out in a specialized immunological laboratory.

**Polymorphism of blood leukocyte acid phosphatase.** Polymorphism of blood leukocyte acid phosphatase was assayed electrophoretically in agarose gel as described previously (9).

**Blood plasma protein fractions.** Major protein fractions in blood plasma were assayed with Cormay Gel Protein 100 kits and the procedure supplied by the producer (CORMAY). The total protein content in blood plasma (g/L) and the densitometric measurement of electrophoretic fractions (%) were used in the calculations of the particular protein fractions (g/L) concentrations.

**Statistical analysis of data.** The results obtained were subjected to the data distribution fit test with the normal distribution curve. The effect of the AcP polymorphism in blood leukocytes (phenotype A and phenotype AB), natural BLV infections (EBL+ and EBL- cows) and month of lactation (1, 2, and 3 months after calving) on the composition of blood plasma protein fractions (three-factorial variance analysis) was analysed. Moreover, the effect of the interaction between these factors on the level of the protein fractions were analysed in the EBL-positive and EBL-negative cows (two-factorial variance analysis). The general ANOVA/MANOVA for the factor systems and POST HOC TEST and Scheffé method were applied. Calculations were made with STATISTICA 6.0 computer software.

Results

The diagnostic tests applied identified 46 (71.9%) EBL+ and 18 (28.1%) EBL- cows. While analysing blood leukocyte acid phosphatase polymorphism, the A phenotype was found in 16 cows (25.0%), while AB phenotype in 48 cows (75.0%). Within the A phenotype cow group, 11 animals (68.7%) were EBL+ and 5 animals (31.3%) EBL-, while within the AB phenotype cow group, 35 animals (72.9%) were EBL+ and 13 cows (27.1%) EBL-.

While analysing the effect of the blood leukocyte acid phosphatase polymorphism on the blood plasma protein profile, differences in the levels of β1- and β2-globulins and γ-globulins between cows with different AcP phenotypes were found (Table 1). In the A phenotype cows, higher concentrations of β2-globulins (P≤0.05) and γ-globulins (P≤0.01) as well as lower percentage of β1-globulins (P≤0.05) were recorded compared with the AB phenotype cows. The concentrations of the remaining protein fractions were similar in both phenotype groups. A quantitative diversification in some electrophoretic fractions was also recorded between the BLV-positive and the clinically healthy cows (Table 1). In the EBL-positive cows, higher concentrations of β1-globulins (g/L and %) (P=0.11 and P=0.01, respectively) as well as lower concentrations of β2-globulins (P=0.06 and P=0.11, respectively) and γ-globulins (P=0.05 and P=0.05, respectively) were recorded compared with the EBL-negative cows. The concentrations of the remaining protein fractions were similar in both animal groups. Moreover, a significant (P=0.01) effect of the interaction between the AcP polymorphism and BLV infection on the γ-globulin concentration was found (Table 1). It was reflected in the diversification in the level of this fraction between the AcP phenotypes among the EBL-negative specimens (Fig. 1) and in a similar level of this fraction in the EBL-positive cows (Fig. 2). Higher γ-globulin (P≤0.001) concentrations were registered in the blood plasma of the A phenotype clinically healthy cows than in the blood plasma of the AB phenotype cows. Although the quantitative changes related to the BLV infection were also observed for albumins and β1- and β2-globulins (Figs 1, 2), the interaction between the AcP polymorphism and leukaemia was statistically insignificant (Table 1). Moreover, differences in the concentration of β1-globulins and γ-globulins were recorded between the A phenotype leukaemic and clinically healthy cows (Figs 1, 2). In the leukaemic cows, lower levels of γ-globulins (16.4 g/L and 18.0%) and β1-globulins (17.3 g/L and 18.3%) were registered in comparison to the clinically healthy cows with the same phenotype (22.0 g/L and 21.9%, 20.6 g/L and 20.5%, respectively).
While analysing the changes in the concentration of the particular blood plasma protein fractions in the first trimester of lactation, the quantitative diversification in the β₁-globulin and γ-globulin fractions as well as small and statistically insignificant differences in the concentration of other protein fractions were found (Table 1). The highest concentration of γ-globulins was recorded in the first and the lowest in the third month of lactation (P≤0.01 and P≤0.01, respectively). An opposite tendency of changes was observed for β₁-globulins whose concentration in the blood plasma was the lowest in the first and the highest in the third month of lactation (P≤0.01 and P≤0.01, respectively).

Table 1

| Fractions of protein measured | Phases of Phases of Phases of Phases of Phases of AcP Result of diagnostic test Months of lactation Interactions |
|-----------------------------|-------------------|------------------|-----------------|-----------------|----------------|
|                             | A                | AB               | EBL+            | EBL-            | I               | II              | III             | 1x2x3          |
| Albumins (%)                | 36.2             | 36.8             | 36.6            | 36.9            | 36.4            | 36.3            | 37.2            |                |
| α₁-globulins (%)            | 7.5              | 7.3              | 7.4             | 7.2             | 6.8             | 7.4             | 7.9             |                |
| α₂-globulins (%)            | 7.2              | 6.9              | 7.0             | 6.9             | 6.7             | 7.1             | 7.2             |                |
| β₁-globulins (%)            | 7.2              | 7.6              | 7.6             | 7.2             | 7.0<sup>AB</sup> | 7.5<sup>A</sup> | 8.0<sup>A</sup> |                |
| β₂-globulins (%)            | 18.4<sup>a</sup> | 17.2<sup>a</sup> | 17.3            | 18.2            | 17.0            | 16.8            | 18.7            |                |
| γ-globulins (%)             | 18.5<sup>c</sup> | 16.6<sup>c</sup>| 16.8<sup>d</sup>| 17.9<sup>ad</sup>| 18.3            | 16.5            | 16.5            |                |

Mean values denoted with the same small letter are significantly different at P≤0.05. Mean values denoted with the same capital letter are significantly different at P≤0.01; 1x2: the interaction of AcP polymorphism and result of diagnostic test; 2 x 3: the interaction of result of diagnostic test and lactation months; 1x 2x3: the interaction of AcP polymorphism and result of diagnostic test and lactation months *P≤0.05; **P≤0.01.
**Fig. 1.** Blood leukocyte acid phosphatase polymorphism and blood plasma protein profiles in EBL-negative cows (mean ± SD) *P*≤0.05; ***P*≤0.001.
Discussion

Electrophoresis in agarose gel is a common blood plasma protein analysis method. It permits protein separation into 6 fractions and their densitometric measurement. The particular electrophoretic fractions contain several individual proteins whose concentration may fluctuate at different physiological and pathological states. However, the quantitative changes in the electropherogram may be registered only when the normal concentration of these proteins in blood plasma is relatively high. A relationship between the blood leukocyte acid phosphatase polymorphism and the concentration of γ-globulins and β₁- and β₂-globulins was observed (Table 1). In the A phenotype cows, a significantly higher concentration of γ-globulins and β₂-globulins as well as a lower concentration of β₁-globulins were recorded compared with the AB phenotype cows. The γ-globulin fraction is mainly comprised of immunoglobulins IgG, IgM and IgA which move along with the β₂-globulin fraction. Immunoglobulins are synthesised by plasmocytes produced as the result of B lymphocyte activation by various antigens. Within the γ-globulin fraction there is also the C-reactive protein (CRP) which is an element of innate immunity and belongs to the acute-phase proteins. These proteins play a wide range of biological functions and their role in the acute-phase response (APR) involves counteracting the spreading of the inflammatory process and limiting tissue damage (17, 20). Interleukine 6 (IL-6) plays a key role in the regulation of the acute-phase protein synthesis (8, 20). It is a pleiotropic cytokine involved in the regulation of immune responses, APR, and haematopoiesis (1). In addition, IL-6 affects the activated B lymphocytes and plays the main role in the transformation of these cells...
into plasmocytes. Moreover, it participates in the activation of the T lymphocytes and their differentiation into the T cytotoxic lymphocytes. Higher γ-globulin concentration registered in the blood plasma of the A phenotype cows seems to indicate the occurrence of a stronger immunological reactions in these animals than in the AB phenotype cows. This seems to be connected with the occurrence of a significantly greater population of the B lymphocytes (9) and greater proliferation efficiency of these cells in these specimens (12). As a result, the B lymphocytes in the A phenotype cows are more easily activated and transformed into plasmocytes synthesising immunoglobulins than those of the AB phenotype cows. The above-mentioned properties may result in greater concentrations of γ-globulins in blood plasma of the A phenotype cows caused by higher level of immunoglobulins. This hypothesis seems to be confirmed by the higher concentration of β2-globulins also recorded in these cows (Table 1). These globulins also include part of immunoglobulins. Moreover, the component of the C3 complement, occurs within the β2-globulins, and is an important element of the complement system, which, in turn, is one of the principle mechanisms of innate immunity. The concentration of this protein is the highest among all the proteins of the complement system. Moreover, a significantly lower percentage of β1-globulins were registered in blood plasma of the A phenotype cows (Table 1). This fraction is mainly comprised of transferrin and β-lipoprotein (bound with LDL) whose concentration change may have an effect on its content in the electropherogram. It is currently difficult to interpret the observed correlations, which require further analyses.

While comparing the composition of the blood plasma electrophoretic fractions between the leukemic and the clinically healthy cows, different concentrations of the same fractions exhibiting the relationship with the AcP polymorphism were found. However, the trend of quantitative changes was opposite to that found in animals with different AcP phenotypes (Table 1). In the EBL+ cows, a higher concentration of β1-globulins (g/L and %) (P≤0.01, P≤0.01, respectively) as well as lower concentration of β2-globulins (P=0.07 and P=0.11, respectively) and γ-globulins (P≤0.05 and P≤0.05, respectively) were recorded compared with the clinically healthy animals. BLV infection development in cattle evokes changes in the lymphatic system and expansion of immature B lymphocytes (16). This leukosis form is called persistent lymphocytosis (PL) and persists in an infected animal for many years. Lymphocytosis is not always accompanied by BLV infection. In some infected animals, an aleukaemic form (BLV+PL-) occurs with regular or slightly lower populations of lymphocytes (4). Moreover, in a small percentage of BLV+ animals lymphosarcoma - a neoplastic form develops and causes animal death. The results of immunoglobulin concentration in BLV infected cattle are ambiguous. Some authors recorded significant differences in the blood plasma IgM level between BLV+ with PL, BLV+PL- and BLV-free cattle (7, 16, 21). Other authors observed small and statistically insignificant differences (19, 22). On the other hand, the blood plasma IgG level was not very much different in these animals (7, 21, 22). Moreover, a negative correlation was recorded between the number of the B lymphocytes and blood plasma IgM concentration in the BLV infected animals (19, 21). Lower concentrations of γ-globulins and β2-globulins in blood plasma of the EBL+ cows registered in this study may have been caused by a dysfunction of the B lymphocytes typical of the PL stage and a lower IgM synthesis. Moreover, an effect of the interaction between the AcP polymorphism and leukosis on the γ-globulin concentration was found (Table 1). This interaction was reflected in the quantitative diversification in the level of this electrophoretic fraction between the different AcP phenotypes, but only among the clinically healthy specimens (Fig. 1), whereas in the leukaemic cows the γ-globulin level was very similar (Fig. 2). Within the A phenotype EBL-negative cows, the concentration of this fraction was higher than that in the EBL-positive cows carrying the same phenotype. Although similar changes between the AcP phenotypes related to the BLV infection were observed for β2-globulins (Figs 1, 2), the interaction between the AcP polymorphism and leukosis was statistically insignificant (Table 1). The obtained results seem to indicate a possible modification of natural reactions between the animal genotype and the processes occurring in the cell by viral proteins present in the lymphatic system of the BLV infected cows.

A significantly higher concentration of β1-globulins recorded in the leukaemic cows makes interpreting the results difficult (Table 1). The concentration of this electrophoretic fraction is largely determined by the level of transferrin. This protein is classified as a non-specific humoral factor as well as an acute-phase protein. Transferrin is the main iron carrier. It transfers Fe2+ ions to the cells in demand of this element and helps the organism to achieve homeostasis previously disturbed by undesirable factors (6). It could be assumed that a higher concentrations of the β1-globulin fraction in blood plasma recorded in the EBL+ cows are connected with the disturbed organism homeostasis caused by BLV infection and development of enzootic bovine leukosis. A quantitative diversification in the β1-globulin fraction was also found between the analysed months of lactation (P≤0.01) (Table 1). The lowest concentration of this fraction was observed in the first and the highest in the third month of lactation regardless of BLV infection. At this research stage it is difficult to interpret the results adequately. It could be assumed that the observed changes are caused by a lower level of transferrin recorded by other authors in cows in their first months after calving (18). The obtained results indicate that more detailed studies into the β1-globulin individual fractions should be performed. Moreover, a diversification in the concentration of blood plasma γ-globulins in the experimental cows was also found but the trend of these changes was different than for the β1-globulin fraction. The highest concentration of this fraction was observed in the first and the lowest in the third month of lactation.
regardless of BLV infection (Table 1). It seems that the observed changes in the γ-globulin concentration are connected with the changes in the hormonal profile occurring in the post-calving period involving the initiation of lactation and intensive synthesis of immunoglobulins, especially IgG. These antibodies are transferred from blood into the mammary gland and, being present in colostrum and milk, they determine the immunity of a calf in its initial life period.

It was found that the blood leukocyte acid phosphatase polymorphism and BLV infection significantly diversify the levels of β1- and β2-globulins and γ-globulins. Considering the essential immunological functions of the proteins constituting these fractions, the recorded relationships indicate different immunological conditions of the specimens carrying different Acp phenotypes and different health status. Moreover, the BLV seems to modify the effect of the blood leukocyte acid phosphatase genotype on the β1- and β2-globulin fractions. The obtained results encourage further, more detailed studies into the individual proteins of the β1- and β2- globulin and γ-globulin fractions in the blood plasma of animals of different phenotypes of acid phosphatase and at different stages of enzootic bovine leukaemia development.

References