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Polymorphism and expression of the tumour necrosis factor-alpha (TNF-alpha) gene in non-infected cows and in cows naturally infected with the bovine leukaemia virus (BLV)

B. Bojarojc-Nosowicz, P. Brym, E. Kaczmarczyk, A. Stachura, A.K. Habel

Department of Animal Genetics, University of Warmia and Mazury, Olsztyn, Poland

ABSTRACT: The objective of this study was to determine the influence of TNF-alpha gene polymorphism at position –824 A > G on TNF-alpha gene expression in BLV-positive animals with aleukaemic (AL) and persistent lymphocytosis (PL) forms of enzootic bovine leukosis (EBL) and in BLV-negative cows. Polymorphism of the TNF-alpha gene at position –824 A > G had a complex influence on TNF-alpha gene expression in cows infected with BLV. In animals with various TNF-alpha genotypes, mRNA levels were stable and similar, but significant differences were noted in the percentages of PBMCs expressing membrane TNF-alpha (mTNF-alpha) protein (PBMCs that were positive for mTNF-alpha). In healthy cows, significant differences in TNF-alpha gene expression were not noted at any of the analysed levels. BLV infection and EBL progression resulted in differential TNF-alpha gene expression at both mRNA and protein levels, but the differences in the amount of the transcript and the percentages of mTNF-alpha+ cells exhibited a reverse trend. The lowest mRNA levels and the highest percentage of PBMCs expressing mTNF-alpha protein were determined in BLV-positive cows. These complex results regarding TNF-alpha gene expression in BLV-positive cows could be attributed to the presence of virus or viral protein/proteins modifying the expression of the TNF-alpha gene.

Keywords: TNF-alpha gene; polymorphism; gene expression; mTNF-alpha; BLV; EBL

The bovine leukaemia virus (BLV) is an aetiological agent of enzootic bovine leukosis (EBL), which is a chronic neoplastic disease. BLV is closely related to HTLV-1 (Human T-lymphotropic Virus type I), which is responsible for leukaemogenesis of T lymphocytes in humans (Aida et al. 2013). The pathogenesis of disease induced by HTLV-1 is still unknown, but certain similarities with BLV have been reported (Aida et al. 2013). Cows with EBL could be a useful animal model for studying the pathogenesis of disease caused by HTLV-1.

Resistance or susceptibility to BLV infection is associated with hereditary traits (Lewin and Bernoco 1986) and host immune responses (Kabeya et al. 2001), but the mechanism of leukaemogenesis induced by BLV is still unclear. Previous studies have demonstrated that a single nucleotide polymorphism (SNP) at position –824 A > G of the tumour necrosis factor-alpha (TNF-alpha) gene in cattle (Konnai et al. 2006a) as well as TNF-alpha mRNA expression in BLV-positive animals could contribute to the pathogenesis of EBL (Kabeya et al. 1999; Amills et al. 2004). Expression of the TNF-alpha gene is regulated by the promoter and 3'UTR regions (Han et al. 1991). SNPs located in the TNF-alpha regulatory region can influence gene expression by changing transcription factor binding sites, such as those for nuclear factor-kappa B (NF-kB) and activator protein-1 (AP1) (Leitman et al. 1991). They can also affect the quantity and stability of mRNA and the translational efficiency of TNF-alpha protein (Sennikov et al. 2014).

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The aim of this study was to determine the relationship between polymorphism of the TNF-alpha gene at position –824 A > G and its expression in cows naturally infected with BLV.

MATERIAL AND METHODS

Materials. The study was performed on Polish Holstein-Friesian cows (n = 127) from three herds free of tuberculosis and brucellosis. The number of animals used in the study varied depending on the type of analysed factor/factors, and is specified in the figures and tables in which the findings are presented. Blood samples for the isolation of peripheral blood mononuclear cells (PBMCs) and genomic DNA were obtained from the mammary vein and collected into tubes containing EDTA. BLV infections were diagnosed by the immunofluorescence method (IMF) based on the presence of p24 protein in PBMCs and using a nested PCR method that detects proviral DNA. Individuals that tested positive for BLV in both methods were considered as BLV-positive animals and were used in the experiment. These cows were further subdivided into two groups: cows with the aleukaemic form (AL) and animals with persistent lymphocytosis (PL).

Total RNA for the measurement of TNF-alpha gene expression at the transcript level was isolated from whole peripheral blood sampled into tubes with a stabilizer (PAXgene Blood RNA Tube-PreAnalytiX, Switzerland). TNF-alpha gene expression in BLV + AL and BLV + PL cows as well as in BLV-negative animals was analysed using a qRT-PCR method. The expression analysis was based on cDNA from BLV + AL, BLV + PL and BLV-negative cows with A/A, G/G and A/G TNF-alpha genotypes in each group (n = 41).

PBMCs isolated at monthly intervals (blood samples were collected twice or three times from each animal) were subjected to viral protein p24 and mTNF-alpha immunolabelling using the IMF method.

Diagnosis of BLV infection

Nested PCR. Genomic DNA was isolated from peripheral blood leukocytes using the Master Pure^{TM} Purification Kit in accordance with the manufacturer's instructions (Epicentre Biotechnologies, USA). The quantity and quality of isolated DNA was evaluated using spectrophotometry (GeneQuant, Pharmacia) and 1% agarose gel electrophoresis according to standard protocols. The composition of the reaction mixture and the thermal cycling profile of double amplification of the *env* viral gene were as described by Markiewicz et al. (2003). The sequence of the used primers has been described by Klintevall et al. (1994) (Sigma, USA). A fragment of the *env* gene of the provirus (427 bp) amplified in PCR I constituted the template for PCR II where the resulting product had the size of 341 bp.

Immunofluorescence (IMF) method

Isolation and preparation of PBMCs for labelling. PBMCs were isolated from whole peripheral blood by density gradient centrifugation using Histopaque-1077, according to the manufacturer's instructions (Sigma, USA). The cells were isolated for immunofluorescent staining according to a previously described procedure (Kaczmarczyk et al. 2004). The isolated cell suspensions had a density of 2×10^6 cells/ml and a minimum purity of 98%.

Identification of BLV-positive cells. BLV-positive cells were identified by indirect IMF with a mouse anti-bovine monoclonal BLV3 antibody (anti-BLVp24, IgG1-VMDR Inc. Pullman, USA) that detects the p24 epitope (main BLV capsid protein) and a goat anti-mouse IgG (H + L) secondary antibody labelled with the PE fluorochrome (Phycoerythrin, orange signal) (Invitrogen, USA) (Kaczmarczyk et al. 2008). The control underwent the same procedure, but without the primary antibody. Smears were stored at +4 °C and were analysed under a fluorescence microscope (Axiolab-Zeiss, Germany) equipped with an appropriate filter, at 1000 × magnification. Cells expressing p24 protein were indicative of BLV infection, whereas cows whose cells did not express the viral protein in any of the examined samples were regarded as free of the infection. The presence of BLV-infected cells was determined based on the analysis of 600 lymphocytes from the blood smear.

Haematological analysis. Total leukocyte counts and lymphocyte percentages were determined in a specialist haematological laboratory based on the results generated by an automatic blood analyser and MGG-stained smears. The above parameters were used to calculate absolute lymphocyte counts

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and identify BLV + AL and BLV + PL individuals. BLV-positive cows with leukocyte counts < 12 × 10^9/l and lymphocyte counts < 8 × 10^9/l were diagnosed as aleukaemic and cows with leukocyte counts > 12 × 10^9/l and lymphocyte counts > 8 × 10^9/l were designated as suffering from persistent lymphocytosis.

Polymorphism of the TNF-alpha gene. Polymorphism of the TNF-alpha gene at position –824A > G was determined using PCR-RFLP/Sac I as described previously (Bojarojc-Nosowicz et al. 2011). Three genotype groups were identified: A/A, A/G and G/G. Genotype A/A was characterised by two DNA restriction fragments with sizes of 168 bp and 81 bp, the G/G homozygote had a single 249 bp fragment, whereas the A/G heterozygote had three gene fragments with sizes of 249 bp, 168 bp and 81 bp.

Expression of the TNF-alpha gene at the mRNA level

RNA isolation. RNA was isolated with the use of the PAXgene^{TM} Blood RNA Kit (PreAnalytiX, Switzerland) in accordance with the manufacturer's instructions. The quantity and purity of the isolated RNA was evaluated with a NanoDrop ND 1000 spectrophotometer (Thermo Scientific, USA). RNA quality was assessed using the Agilent RNA 6000 Nano Kit and Agilent 2100 Bioanalyzer. Samples with RIN (RNA Integrity Number) values higher than 8.5 were used for RNA expression profiling.

cDNA synthesis. cDNA was synthesised with 1 µg of total RNA and the Transcriptor High Fidelity cDNA Synthesis Kit in accordance with the manufacturer's instructions (Roche). Moloney murine leukaemia virus reverse transcriptase (M-MLV RT) and oligo$(dT)_{18}$ primers were used. After synthesis, cDNA was diluted, aliquoted and stored at –20 °C until qRT-PCR analysis.

Real-time quantitative reverse transcription PCR (qRT-PCR). TNF-alpha mRNA was quantified and normalised according to MIQE guidelines (minimum information for publication of quantitative real-time PCR experiments) (Bustin et al. 2009). qRT-PCR was performed with the use of LightCycler 480 SYBR Green I Master reagents in accordance with the manufacturer's instructions (Roche). Two reference genes were used: RPLP0 (ribosomal protein large P0) and UCHL5 (ubiquitin carboxyterminal hydrolase L5). According to Brym et al. (2013), the above genes are characterised by stable expression in the blood cells of both BLV-positive and BLV-negative cows. The sequences of qRT-PCR primers used and details of the amplification conditions are presented in Table 1.

qRT-PCR was performed with the use of 100-fold diluted cDNA in 96-well plates (Roche) in the LightCycler®LC 480 II system (Roche). The amplification of PCR products and melting curve analysis were performed as described previously (Brym et al. 2013). Briefly, cycling conditions consisted of pre-denaturation at 95 °C for 5 min followed by 45 cycles of 95 °C for 10 s, annealing (for temperature, see Table 1) for 10 s and elongation at 72 °C for 10 s. Melting of amplicons was executed by heating to 95 °C for 5 s, cooling to 65 °C for 1 min followed by stepwise increases in tempera-

Table 1. Characterisation of PCR products analysed in the study

Gene ID	Gene name	Primer sequences (forward/reverse), (5'-3') (references)	Annealing temp. (°C)	Tm (°C)	Amplicon size (bp)	E	Error
TNF-alpha	tumor necrosis factor-alpha	F-CTGGTTCAGAACTCAGGTCCT R-GAGGTAAAGCCCGTCAGCA (Donaldson et al. 2005)	58	86	178	1.991	0.005
RPLP0	ribosomal protein large P0	F-CAACCCTGAAGTGCTTGACAT R-AGGCAGATGGATCAGCCA (Donaldson et al. 2005)	58	86	227	1.994	0.002
UCHL5	ubiquitin carboxyl-terminal hydrolase L5	F-ACAAAGACAACTTGCTGAGGAACCC R-GGCAACCTCTGACTGAATAGCACTT (Brym et al. 2013)	60	78	79	2.036	0.002

Tm = melting temperature of amplicon; E = efficiency of qRT-PCR reaction; Error = accuracy of result estimation based on the standard curve

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ture from 65 to 97 °C at a ramp rate of 0.11 °C/s with continuous monitoring of the fluorescence. Furthermore, amplicon specificity was also confirmed by PCR product sequencing and 2% agarose – ethidium bromide electrophoresis. Reaction efficiency (E) for each amplicon was calculated based on a standard curve of 5-point serial dilutions and was determined to be in the range of 1.991–2.036 (Table 1). The qRT-PCR assays were performed in two replicates for each sample target gene and endogenous controls and in three replicates for the negative control (no template control). The quantification cycle (Cq) was determined in LightCycler® 480 SW 1.5 software. The relative quantification of TNF-alpha gene expression was performed with the Relative Expression Software Tool (REST 2009 ver.2.0.13) (Pfaffl et al. 2002), using G/G genotype and BLV-negative cows as a control groups.

Immunophenotypic analysis of mTNF-alpha expression. TNF-alpha expression was evaluated in PBMCs isolated from whole peripheral blood in accordance with the procedure described by Kaczmarczyk et al. (2004). The cell suspension had a density of 2×10^6 cells/ml. The indirect IMF method was used. The mTNF-alpha protein epitope was identified with the use of a mouse anti-bovine monoclonal IgG2b antibody at a working concentration of 0.5 µg/50 µl (Acris GmbH, Germany), biotinylated goat anti-mouse immunoglobulin as the secondary antibody (Dako Cytomation, Denmark) and streptavidin conjugated with Texas Red fluorochrome (red signal) (Vector Lab. Inc., USA). The control underwent the same procedure, but without the primary antibody.

Smears were stored at +4 °C and were analysed under a fluorescence microscope (Axiolab-Zeiss, Germany) equipped with an appropriate filter, at 1000× magnification. Six hundred cells were registered per smear, and the percentage of PBMCs expressing mTNF-alpha protein was observed.

Statistical analysis. The influence of TNF-alpha gene polymorphism (factor 1) and BLV infection (factor 2) on mRNA levels and the percentages of PBMCs expressing mTNF-alpha were analysed.

Data relating to TNF-alpha gene expression in PBMCs were processed in view of the arithmetic mean, standard error of the mean (SEM) and data distribution fit test. The Kruskal-Wallis non-parametric test and a median test for comparing specific traits were used when the distribution of the analysed values was not consistent with normal distribution (including mathematically transformed data). Differences between groups were verified by multiple comparisons in a non-parametric test at $P < 0.05$, $P < 0.01$ and $P < 0.001$. The results were processed in the Statistica 10.0 program.

RESULTS

TNF-alpha gene polymorphism and BLV infections in the analysed animals

Analyses of TNF-alpha gene polymorphism at position –824 A > G in the studied herds revealed three genotypes: A/A in thirty-three individuals (26.0%), A/G in forty-eight cows (37.8%) and G/G in forty-six individuals (36.2%). A total of seventy-eight BLV-positive (BLV+) and forty-nine BLV-negative (BLV–) cows were identified. In the group of BLV+ animals, sixty-three individuals (83.3%) were diagnosed with BLV + AL, and fifteen cows (16.7%) with BLV + PL.

The effect of TNF-alpha gene polymorphism on its expression at the mRNA level and the percentages of mTNF-alpha+ cells

The raw Cq values estimated for TNF-alpha and reference genes with regard to TNF-alpha genotypes and BLV infection are shown in Table 2. The analysis of associations between polymorphism of the TNF-alpha gene and its expression at the mRNA level revealed somewhat higher values in BLV-negative cows with the A/A genotype in comparison with control animals with the G/G genotype, but the noted differences were not statistically significant ($P = 0.51$) (Figure 1). In A/G heterozygotes, TNF-alpha gene expression was similar to that in control G/G homozygotes (Figure 1). In BLV+ cows, differences in mRNA levels were observed in animals with A/A and A/G genotypes in comparison to the control group (G/G), but they were not statistically significant (Figure 1).

The percentage of PBMCs expressing mTNF-alpha protein was also analysed, and statistically significant differences were found between TNF-alpha genotypes in BLV+ animals. The highest percentage of mTNF-alpha+ cells was noted in G/G homozygotes, and the lowest – in A/G heterozygotes ($P < 0.001$) (Figure 2). In BLV-negative cows,

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Table 2. The raw Cq values estimated for TNF-alpha and reference genes with regard to TNF-alpha genotypes and BLV infection

Cq values	Cows					
	BLV+			BLV–		
	Genotypes					
	AA $n = 9$	AG $n = 10$	GG $n = 8$	AA $n = 4$	AG $n = 5$	GG $n = 5$
TNF-alpha						
Mean Cq	26.14	26.06	26.76	25.38	25.31	26.33
Range of Cq	24.94–28.73	24.89–28.05	24.46–29.29	24.06–26.99	24.27–27.53	25.08–27.98
SEM	0.43	0.33	0.59	0.51	0.55	0.67
RPLP0						
Mean Cq	22.92	22.62	22.49	23.00	22.25	23.39
Range of Cq	21.96–23.67	21.68–23.49	22.01–23.45	22.67–23.14	21.78–22.74	22.31–24.63
SEM	0.11	0.16	0.13	0.09		0.36
UCHL5						
Mean Cq	25.96	26.02	25.75	25.98	25.81	26.07
Range of Cq	25.20–26.42	25.58–26.25	24.80–26.14	25.54–26.41	25.24–26.17	25.85–26.31
SEM	0.08	0.06	0.17	0.14	0.16	0.09

the differences between TNF-alpha genotypes were small and not statistically significant (Figure 2).

The influence of BLV infection and EBL progression on TNF-alpha mRNA levels and the percentages of mTNF-alpha+ cells

The raw Cq values obtained for TNF-alpha and reference genes in BLV-negative and BLV-positive animals are presented in Table 3.

The analysis of associations between BLV infection stage and TNF-alpha mRNA expression levels revealed significant differences ($P < 0.05$) between BLV– and BLV+ cows (Figure 3). The expression of TNF-alpha was 2.2-fold higher in BLV-negative animals in comparison to BLV + PL individuals.

Significant differences between healthy and BLV+ cows were also observed in the proportion of mTNF-alpha+ immunophenotyped cells, but the differences between those groups was reversed compared to that noted at the mRNA level

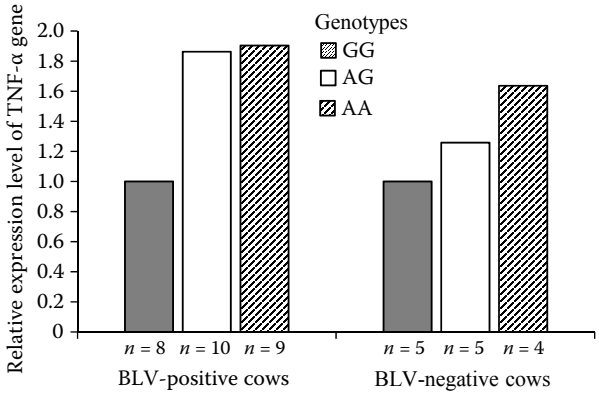

Figure 1. Relative levels of gene expression in BLV– and BLV+ cows with various TNF-alpha genotypes. The results are presented as the ratio of gene expression levels in the analysed groups in comparison with the G/G genotype as the control group

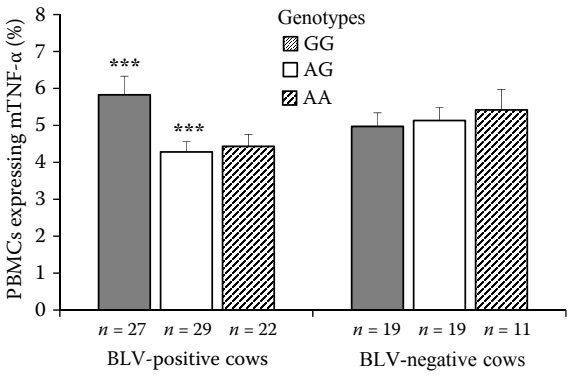

Figure 2. Percentages of PBMCs expressing mTNF-alpha in BLV– and BLV+ cows with various TNF-alpha genotypes (mean ± SEM). SEM = standard error of the mean; ***$P < 0.001$

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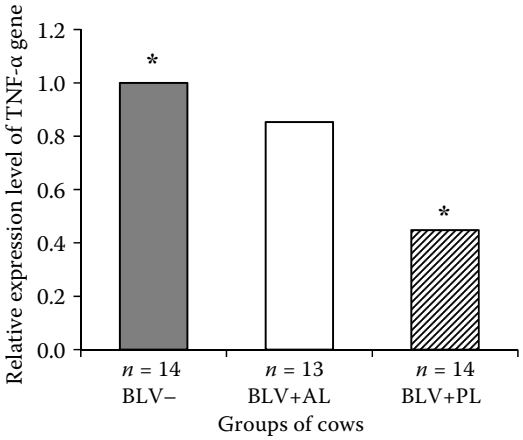

Figure 3. Relative levels of TNF-alpha gene expression in BLV– and BLV+ cows. The results are presented as the ratio of gene expression levels in the analysed groups in comparison with BLV– cows as the control group. *$P < 0.05$

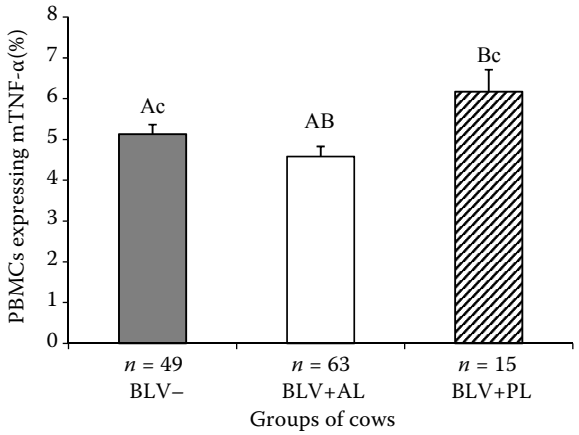

Figure 4. Percentages of PBMCs expressing mTNF-alpha in BLV– and BLV+ cows (mean ± SEM). SEM = standard error of the mean. Mean values followed by the same capital letters or lowercase letters are significantly different at $P < 0.001$ or $P < 0.05$

(Figure 4). The highest percentage of mTNF-alpha+ cells was reported in BLV + PL animals, and the lowest – in BLV + AL cows ($P < 0.001$). In healthy cows, the percentage of mTNF-alpha+ cells was higher than in BLV + AL individuals, but lower than in BLV + PL cows ($P < 0.05$) (Figure 4).

DISCUSSION

The influence of TNF-alpha gene polymorphism at position –824 A > G on TNF-alpha gene expression at the mRNA level and the development of BLV infections remains poorly investigated (Konnai et al. 2006a). Low or undetectable mRNA levels were reported in PBMCs isolated from BLV+ cows with various TNF-alpha genotypes. Higher and detectable expression levels of the TNF-alpha gene were observed *in vitro* when cells were stimulated with the mitogen Concanavalin A (Konnai et al. 2006a).

In the present study, the transcript was acquired in the amount sufficient for analysing the expression of the TNF-alpha gene. qRT-PCR was performed on RNA isolated from peripheral blood cells whose proliferative activity was similar to that noted *in vivo* (not stimulated with mitogens).

Table 3. The raw Cq values obtained for TNF-alpha and reference genes in BLV-negative and BLV-positive animals

Cq values	Groups of cows		
	BLV– *n* = 14	BLV+AL *n* = 13	BLV+PL *n* = 14
TNF-alpha			
Mean Cq	25.69	25.84	26.72
Range of Cq	24.06–27.98	24.46–28.73	24.94–29.29
SEM	0.34	0.33	0.36
RPLP0			
Mean Cq	22.87	22.74	22.63
Range of Cq	21.78–24.63	22.01–23.67	21.68–23.45
SEM	0.19	0.26	0.11
UCHL5			
Mean Cq	25.95	25.91	25.92
Range of Cq	25.24–26.41	24.80–26.42	25.20–26.22
SEM	0.08	0.13	0.05

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Normalisation of gene expression levels is a critical factor in qRT-PCR data analysis (Valceckiene et al. 2010; Falkenberg et al. 2011). It has been recommended that at least two reference genes should be used in qRT-PCR analyses of expression (Bustin et al. 2009). Most published data regarding changes in gene expression in BLV-infected animals were normalised using only one reference gene, usually the β-actin gene (ACTB) (Konnai et al. 2006b). A recent study indicated that ACTB and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were not suitable reference genes for analyses of gene expression in the peripheral blood cells of BLV-infected cows (Brym et al. 2013). The authors demonstrated that ACTB and GAPDH were characterised by the lowest stability in the group of 10 tested reference genes. It was revealed that the gene pairs of ribosomal protein large P0 (RPLP0) and ubiquitin carboxyterminal hydrolase L5 (UCHL5) as well as beta-2 microglobulin (B2M) and RPLP0 are characterised by the highest stability of expression in the analysed biological material and fulfil reference gene requirements (Brym et al. 2013). Thus, RPLP0 and UCHL5 genes were used in this study. The differences between the mRNA levels noted in our study and those reported by Konnai et al. (2006b) could be attributed to different methodological approaches.

In the current experiment, the analysis of correlations between TNF-alpha gene polymorphism at position –824 A > G and mRNA levels revealed non-significant differences between TNF-alpha genotypes among both healthy and BLV-infected animals. Significant differences in TNF-alpha gene expression were observed at the protein level, but only in BLV-infected cows. Animals with the G/G genotype were characterised by a significantly higher percentage of mTNF-alpha+ cells than A/G heterozygotes.

The differences in TNF-alpha gene expression at the mRNA and protein levels in BLV+ animals could be attributed to the influence of viral proteins on gene expression.

The viral Tax protein deregulates the expression of cellular genes (including TNF-alpha, IL-2 and its receptor, Bcl-xl and other) and immortalises cells infected with HTLV-1 (Hajj et al. 2012). The Tax protein of BLV and HTLV-1 can inhibit or induce the synthesis of cellular mRNA to escape the control of the host's immune system (Yoshida 2001; Twizere et al. 2003). Retrovirus activity is also observed in the post-transcriptional control of gene expression. Retroviruses induce the degradation of cellular mRNA to obtain better access to the cell's translational mechanisms, thus disrupting the host's immune processes and inducing neoplastic transformations in cells (Mocquet et al. 2012).

This study also analysed the influence of BLV infection and the severity of EBL on the expression of the TNF-alpha gene. Significant differences in mRNA levels and the percentages of TNF-alpha+ cells were noted between infected and healthy animals, but the differences in gene expression exhibited a reversed trend in those groups. In BLV + PL cows, mRNA levels were 2.2-fold lower than in healthy animals, whereas the percentages of mTNF-alpha+ cells in BLV + PL cows were considerably higher than in healthy individuals. This anti-correlation between expression at the mRNA and protein levels was also reported by other authors (Sarro et al. 2010; Vogel et al. 2010; Schwanhausser et al. 2011). Sarro et al. (2010) observed substantially lower levels of CD20 protein in B lymphocytes sampled from patients with chronic lymphatic leukaemia than in cells isolated from healthy subjects (control groups), whereas the quantity of the transcript was similar in both groups.

Discrepancies in expression between mRNA and protein could be attributed to extracellular and intercellular factors that lead to variations in the half-life of proteins synthesised from the mRNA in eukaryotic organisms (Twizere et al. 2003). Cells can control protein degradation and synthesis rates in a variety of ways by adapting those processes to their own needs (Pratt et al. 2002). BLV and HTLV-1 can collaborate with selected cellular proteins, including RNA helicase A (RHA), and modify gene translation processes in host cells (Bolinger and Boris-Lawrie 2009). RHA has a conserved sequence, and it participates in most processes involving RNA (Jankowsky 2011). Interactions between retroviruses and RNA helicases, including RHA, indicate that retroviruses can directly influence gene translation in the host cells (Hartman et al. 2006). The presence of structural elements that facilitate interactions with the RHA enzyme has been found in BLV in recent years (Bolinger and Boris-Lawrie 2009).

The results of this study indicate that polymorphism of the TNF-alpha gene at position –824 A > G has an complex effect on TNF-alpha gene expression in BLV-infected cows. In animals with different TNF-alpha genotypes, mRNA levels were stable

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and similar, but significant differences were noted in the percentages of PBMCs expressing mTNF-alpha protein. In healthy cows, no significant differences in TNF-alpha gene expression were reported at any of the analysed levels. BLV infection and EBL severity significantly influenced TNF-alpha gene expression at both the mRNA and protein levels. The differences in the amount of the transcript and the percentages of mTNF-alpha+ cells exhibited a reverse trend in healthy and BLV-infected cows.

The relatively ambiguity of the results relating to the expression of the TNF-alpha gene in BLV+ animals could be attributed to the modifying effects of the virus and its protein/proteins on gene expression. Our findings seem to confirm the observations made by other authors (Twizere et al. 2003; Konnai et al. 2006a), which suggest that BLV is a potential cause of discrepancies between expression of a cellular gene at the mRNA and protein levels.

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Corresponding Author:

Barbara Bojarojc-Nosowicz, University of Warmia and Mazury, Faculty of Animal Bioengineering, Department of Animal Genetics, ul. M. Oczapowskiego 5, 10-957 Olsztyn, Poland
E-mail: b.bojarojc@uwm.edu.pl