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EXPRESSION of *NK-lysin*, *blvr*, *ifn-α* AND BLOOD CELL POPULATIONS IN COWS INFECTED BY BOVINE LEUKEMIA VIRUS

G.Yu. KOSOVSKII¹, V.I. GLAZKO^{1, 2}, S.N. KOVAL'CHUK¹, A.L. ARKHIPOVA¹,
T.T. GLAZKO^{1, 2}

¹Center for Experimental Embryology and Reproductive Biotechnology, Federal Agency of Scientific Organizations, 12/4, ul. Kostyakova, Moscow, 127422 Russia, e-mail vigvalery@gmail.com, gkosovsky@mail.ru, s.n.kovalchuk@mail.ru, tglazko@rambler.ru (corresponding author);

²K.A. Timiryazev Russian State Agrarian University—Moscow Agrarian Academy, 49, ul. Timiryazevskaya, Moscow, 127550 Russia

ORCID:

Kosovskii G.Yu. orcid.org/0000-0003-3808-3086

Koval'chuk S.N. orcid.org/0000-0002-5029-0750

Glazko V.I. orcid.org/0000-0002-8566-8717

Glazko T.T. orcid.org/0000-0002-3879-6935

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Abstract

At now, it is impossible to prevent or control the spread of retroviral infections, in particular, bovine leukemia virus (BLV). The existing methods of vaccination and detection of infected animals remain insufficient (G. Gutiérrez et al., 2014; M. Nishiike et al., 2016) which necessitates further investigations of the interactions between pathogen and host organism. Previously, we obtained evidence that a common characteristic for BLV infected animals with moderate and high leukocytosis was the increase in platelets, and in cows with a pronounced leukocytosis the decrease in the number of neutrophils (G.Yu. Kosovskii et al. 2017). In order to assess the relationship between BLV infection in animals, the ratio of cell populations in the peripheral blood and the expression of the genes, encoding the BLV receptor (*blvr* gene), a comparative analysis of antiviral protection protein, interferon alpha (INFA), and effector protein of innate immunity, NK-lysin, was carried out in two groups of cows that differed in origin and farm conditions. In result, the evidences were obtained that cows from two farms differed mainly in the amount of peripheral blood neutrophils and platelets. However, in both farms the BLV infected animals had the reduced gene expression of NK-lysin and an increased number of platelets compared to cows free from infection. Relatively increased expression of *blvr* was observed in BLV infected cows, reflecting, apparently, the increase in the proportion of the young forms of B-lymphocytes (M. Lavanya et al., 2008). On the basis of the own obtained findings and literature data the scheme is proposed of the influence of BLV on the expression of NK-lysin and the suppression of apoptosis. As per the scheme, viral protein TAX (a transcription activator) induces the expression of host *tnf-α* gene (M. Arainga et al., 2012) which, in turn, activates the Treg regulators of immune homeostasis (L.Y. Chang et al., 2015); Treg produces TGF-β (transforming growth factor beta), TGF-β inhibits the proliferation and activity of T-killers and NK-cells, the producers of NK-lysin, and increases the number and activity of platelets which inhibits the apoptosis (K. Ohira et al., 2016; S.C. Tao et al., 2016). The proposed scheme suggested that the key event in pathogenesis, induced by BLV, is the effect on the innate immune system.

Keywords: bovine leukemia virus, granulocytes, agranulocytes, NK-lysin, the receptor of bovine leukemia virus, interferon-alpha, gene expression, innate immunity

Infections are the main cause of economic losses in dairy and beef cattle husbandry. A significant contribution to these is made by enzootic bovine leukosis (EBL) of cattle caused by the Bovine Leukemia Virus (BLV) [1, 2]. Among animals infected by BLV, 60-70 % remain asymptomatic carriers of the pathogen; over time, lymphocytosis develops approximately in 30 % of these carriers, while leukemia and lymphoma occur only in 5-10 % of infected animals. Health improvement of herds

is difficult because antibodies to the virus proteins can be found in animals even in the absence of virus-producing B-lymphocyte clones, and the detection (or non-detection) of the integrated proviral DNA of BLV does not always provide a reliable prognosis for the development of the disease, therefore, there is a need to search for additional characteristics of infectious process induced by BLV [3].

Most often, the BLV proviral DNA is carried by about 1 % of peripheral blood cells [3]. There are two subpopulations of B-lymphocytes infected by BLV, the first subpopulation consists of active producers of mature viral particles and IgM immunoglobulin, and the second one consists of “silent” B-lymphocytes with low expression of BLV proviral DNA and IgM, which differ from the cells of the first subpopulation in higher expression of several oncogenes and in an increased rate of cell death. Both cell subpopulations have the same increased expression of the BLV receptor on B-lymphocytes as compared to that of uninfected animals. That is, the BLV susceptibility does not differ between these two subpopulations [4]. It was found that the number of virus-producing B-lymphocytes is significantly higher in the ex vivo system than in vivo, which indicates the presence of intensive “clearing” selection against BLV-producing B-lymphocytes with involvement of immune system factors [5].

Our previous study of profiles of peripheral blood cell populations in groups of Black-and-White Holstein breed infected with BLV and free from infection indicated a link between pronounced leukocytosis, an increment in the number of platelets, a decrease in neutrophils, and a relatively high neutrophil set enrichment in animals with antibodies to BLV (RID⁺), but without the presence in their genomes of BLV proviral DNA [6].

This paper is the first to report the analysis of gene expression for effector protein NK-lysin (one of the main proteins of cytotoxic T-killer and NK-cell granules) [7], a BLV receptor (BLVR, delta subunit of the AP3 transport system of lymphocyte cytolemma) [8] and monocyte interferon alpha (IFNA) [9], and to suggest a scheme of the BLV impact on the *NK-lysin* expression and suppression of apoptosis.

The aim of the work was to identify factors that could be associated with changes in the functional activity of the immune system in animals infected by BLV.

Techniques. The study included animals aged 2 to 5 years; 57 Black-and-White Holstein cows (ZAO Mozhayskoe, Moscow Province), and 60 crossbreeds of Simmentals × Holsteins obtained by recurrent backcrossing for Holstein breed domination (Zarya farm, Penza Province). Blood samples for analysis were collected from the tail vein.

Erythrocyte and leukocyte profiles and erythrocyte characteristics were determined on an automatic hematological analyzer Abacus junior Vet5 (Diatron, Austria, the principle of operation is based on the Coulter method). Individual samples of fresh whole peripheral blood of each animal (4 ml) stabilized with EDTA were used. Carriers of BLV proviral DNA were detected using Mancini radial immunodiffusion (RID) and PCR protocol developed by us earlier [10]. DNA was extracted from 100 µl of whole blood using a set of M-Sorb reagents for DNA isolation from clinical samples (Synthol, Russia) according to the manufacturer's instruction. Based on this, animals were separated into those free of infection and carrying in BLV proviral DNA the genome. Total RNA was isolated from 1 ml of whole blood sample using ExtractRNA reagents (Eurogen, Russia) in accordance with the manufacturer's recommendations. RNA treatment with DNase I (Thermo Fisher Scientific, USA) and synthesis of the first cDNA chain using the MMLV RT kit (Eurogen, Russia) was performed according to the manufacturers' instruction.

NK-lysin, *blvr*, and *ifn-α* gene expressions was compared by quantitative polymerase chain reaction (RT-PCR) in a LightCycler 96 device (Roche, Swit-

zerland) with the use of SYBR Green intercalating dye and PCR qPCRmix-HS SYBR kit (Eurogen, Russia). The ribosomal protein gene RPLPO expression was the reference. The amplification was carried out in 20 µl of the reaction mixture containing 0.2 µmol of the forward and reverse primers and 2 µl of cDNA. Following primers were used: for *NK-lysin* — 5'-CCTCGGTGCTCCTGGTYGC-3', 5'-GGTCACCCTGGGGATCCTC-3'; for *blvr* — 5'-CTATCGGACCAGTATGTGAAG-3', 5'-CTCCTCGGTGACG-ATGTCC-3'; for *ifn-α* — 5'-GATGAGAGCCTCCTGGACAA-3', 5'-GAAGTATTTCCTCACAGCCAG-3', and for *RPLPO* — 5'-CAACCCTGAAGTGCTTG-ACAT-3', 5'-CAGATGGATCAGCC-AAGAAG-3'. PCR protocol was as follows: denaturation at 95 °C for 15 s, primer annealing at 60 °C for 15 s, elongation at 72 °C for 15 s (40 cycles). The fluorescence recorded in the SYBR fluorescent channel. The reaction specificity was verified based on the temperature dissociation curves of the amplicons obtained.

Quantitative analysis of gene expression was carried out using a Light-Cycler 96 SW1.1 software (Roche, Switzerland).

Statistical data processing was performed in Statistica 6.0 software (StatSoft Inc., USA). Differences were considered significant at $P < 0.05$. The tables show the arithmetic mean ($\bar{X}$) and the errors of the arithmetic mean ($\pm x$).

Results. In our previous studies, evidence has been obtained that cows infected with BLV differ from those free from infection not only by leukocytosis, but also by a reduced counts of neutrophils, as well as an increased amount of platelets and monocytes [6]. Infected animals were divided into two groups by the number of leukocytes, i.e. the cows close to the typical for uninfected animals and those with high leukocytosis (more than $17 \times 10^9/l$).

1. Comparative analysis of peripheral blood erythrocyte and leukocyte profiles in cows free from infection (control) and infected by bovine leukemia virus, in two farms in the Moscow and Penza regions ($\bar{X} \pm x$)

Cell population, characterization	Cows without BLV proviral DNA (RID ⁻ , BLV ⁻ , control)	Cows infected by BLV (RID ⁺ , BLV ⁺)	
		without high leukocytosis	with max leukocytosis
Erythrocytes:			
№ 1	6.86±0.22* (<i>n</i> = 7)	6.15±0.17 (<i>n</i> = 16)	6.34±0.36* (<i>n</i> = 6)
№ 2	5.79±0.29* (<i>n</i> = 10)	5.60±0.29 (<i>n</i> = 7)	5.66±0.27* (<i>n</i> = 8)
Leucocytes:			
№ 1	10.21±0.70 (<i>n</i> = 7)	11.73±0.88 (<i>n</i> = 16)	25.49±1.01** (<i>n</i> = 6)
№ 2	7.75±1.08 (<i>n</i> = 10)	9.00±1.44 (<i>n</i> = 7)	34.21±6.18** (<i>n</i> = 8)
Lymphocytes:			
№ 1	4.85±0.48 (<i>n</i> = 7)	6.12±0.73 (<i>n</i> = 16)	21.70±1.08 (<i>n</i> = 6)
№ 2	4.36±0.94 (<i>n</i> = 10)	5.40±1.27 (<i>n</i> = 7)	29.68±5.71 (<i>n</i> = 8)
Monocytes:			
№ 1	0.52±0.15 (<i>n</i> = 7)	0.28±0.09 (<i>n</i> = 16)	1.61±0.24* (<i>n</i> = 6)
№ 2	0.36±0.11 (<i>n</i> = 10)	0.27±0.10 (<i>n</i> = 7)	0.61±0.21* (<i>n</i> = 8)
Neutrophils:			
№ 1	4.54±0.50* (<i>n</i> = 7)	4.63±0.83* (<i>n</i> = 16)	1.66±0.35* (<i>n</i> = 6)
№ 2	2.39±0.65* (<i>n</i> = 10)	2.76±0.38* (<i>n</i> = 7)	3.31±0.59* (<i>n</i> = 8)
Eosinophils:			
№ 1	0.45±0.16 (<i>n</i> = 7)	0.59±0.09 (<i>n</i> = 16)	0.51±0.18* (<i>n</i> = 6)
№ 2	0.63±0.12 (<i>n</i> = 10)	0.55±0.12 (<i>n</i> = 7)	0.93±0.20* (<i>n</i> = 8)
Basophils:			
№ 1	0.0114±0.0014* (<i>n</i> = 7)	0.0088±0.0027 (<i>n</i> = 16)	0.0067±0.00211* (<i>n</i> = 6)
№ 2	0.0050±0.0022* (<i>n</i> = 10)	0.0143±0.0043 (<i>n</i> = 7)	0.0175±0.0059* (<i>n</i> = 8)
Platelets:			
№ 1	12.90±9.91* (<i>n</i> = 7)	160.81±47.55 (<i>n</i> = 16)	122.33±78.28 (<i>n</i> = 6)
№ 2	126.50±35.15* (<i>n</i> = 10)	318.14±107.92 (<i>n</i> = 7)	252.62±43.35 (<i>n</i> = 8)
The average volume of erythrocytes, μm ³ :			
№ 1	44.86±2.11** (<i>n</i> = 7)	46.12±0.86** (<i>n</i> = 16)	46.50±1.09 (<i>n</i> = 6)
№ 2	55.30±1.58** (<i>n</i> = 10)	52.86±1.12** (<i>n</i> = 7)	49.75±1.42 (<i>n</i> = 8)
Variability of erythrocytes in diameter, %:			
№ 1	20.83±0.52 (<i>n</i> = 7)	18.55±0.34 (<i>n</i> = 16)	18.83±0.31 (<i>n</i> = 6)
№ 2	27.43±9.17 (<i>n</i> = 10)	17.69±0.48 (<i>n</i> = 7)	17.09±0.36 (<i>n</i> = 8)

Note. BLV⁻ and BLV⁺ — the presence or absence of bovine leukemia virus, RID⁻ and RID⁺ — negative and positive reaction of immunodiffusion. Erythrocytes — $\times 10^{12}/l$, leucocytes — $\times 10^9/l$; № 1 and № 2 — the farms surveyed, n — number of animals tested.

* $P < 0.05$, ** $P < 0.01$ (reliability of differences from control).

In the present study, we analyzed the profiles of peripheral blood cells in crossbred cows resulted from recurrent backcrossings of Simmentals with Holsteins (Table 1) in order to clarify the reproducibility of the differences we previously repealed.

The animals of these two farms differed noticeably by the average erythrocyte counts and volume, as well as by the abundance of the populations of neutrophils, basophils and platelets. Interestingly, a relatively larger number of erythrocytes in cows from ZAO Mozhaiskoe (hereinafter, referred to as № 1) coincided with a smaller individual volume of erythrocytes compared to that in animals from the Zarya farm (hereinafter, № 2) (see Table 1). In this, the cows from both farms showed a statistically significant ($P < 0.05$) negative correlation between the number of erythrocytes and their average volume ($r = -0.90$ for № 1 and $r = -0.86$ for № 2). So, apparently, the total functional potential of the erythroid cell series can differ not significantly.

Blood leukocytes, lymphocytes, monocytes and eosinophils in the animal subgroups free from infection and with moderate leukocytosis did not differ between both farms. But in the farm № 2, the number of leukocytes and lymphocytes was statistically significantly higher ($P < 0.05$), and monocytes were lower ($P < 0.05$) in the subgroup with high leukocytosis, when compared to the farm № 1 (see Table 1). Cows from farm № 1 showed some thrombocytopenia in comparison to the animals from farm № 2, with the same differences between subgroups for platelets, i.e. the smallest counts were in animals free from infection, and the largest counts were observed in the animals infected by BLV and moderate leukocytosis (see Table 1).

In cows from farm № 1 which were free from infection (conditionally control subgroup), blood basophils were statistically significantly higher ($P < 0.05$) than that in farm № 2, and if in farm № 1 in animals infected by BLV the number of basophils decreased compared to the conditional control, then in the farm № 2, on the contrary, this index increased (see Table 1).

Different subgroups from farm № 2 did not differ significantly in blood neutrophils, whereas in the farm № 1 in the subgroup with pronounced leukocytosis, the number of neutrophils was the lowest. At the same time, as to neutrophils in the subgroups free from infection and with moderate leukocytosis, the figures in the farm № 1 were almost twice as high as in the farm № 2 (see Table 1). In the scholarly publications, there is conflicting data about the number of neutrophils as impacted by BLV in the carrier animals. Some researchers did not found differences between cows infected and non-infected by BLV [11], while the others report about neutrophilia observed in some animals [12]; also the pronounced neutrocytosis was found in the milk of BLV infected cows [13].

Thus, in cows of different origin, kept in different farms, the leukocyte component, when compared peripheral blood agranulocytes and eosinophils, was similar in the animal free from BLV infection and with moderate leukocytosis, but differed significantly when compared neutrophils and basophils, which were higher in the farm № 1, and platelets, which were lower in the farm № 1. Another manifestation of differences was that in the farm № 1 in subgroups with pronounced leukocytosis, the counts of neutrophils and basophils significantly decreased, if compared to the cows from the farm № 2, at lower scores of leukocytes and lymphocytes (see Table 1). The obtained data indicates that cows from farm № 2 have a definite suppression of cell populations associated with the function of innate immunity (neutrophils, basophils) in comparison to the animals from farm № 1.

In order to evaluate the activity of key genes involved in pathogenesis induced by BLV, we compared expression of *NK-lysin*, *blvr*, and *ifn- α* genes

(Table 2). The *NK-lysin* expression was significantly reduced, more than 2-fold, in animals free from infection from farm № 2, when compared to the same subgroup of cows from farm № 1. This is consistent with the data that in the animals from farm № 2 there is a certain suppression of blood cell populations associated with the innate immunity (see Table 1). Nevertheless, BLV infected animals from farm № 2, alike cows from farm № 1, showed a marked decrease in *NK-lysin* expression (see Table 2). In other words, BLV-infected animals showed further suppression of natural immunity effectors, namely NK-lysin, despite the differences between the farms in patterns of peripheral blood cell populations associated with natural immunity of animals. It should be noted that in both farms the subgroups were identified that had antibodies to BLV (RID⁺), but no cell clones carrying BLV proviral DNA in the genome. There were 7 such cows among 34 RID⁺ animals tested (20.6 %) in the farm № 1, and 11 ones out of 38 tested (28.9 %) in the farm № 2. This is slightly more than that reported [12, 14], but, nevertheless, reproducible in different farms and testifies to the regularity of false-positive results in the detection of animals infected with BLV when using only RID.

2. Expression of genes *NK-lysin*, *blvr*, *ifn-α* in peripheral blood of cows free from infection (control) and infected by bovine leukemia virus (with BLV proviral DNA) ($\bar{X} \pm x$, two farms in Moscow and Penza regions)

Farm, gene	RID ⁻ cows (n) without BLV proviral DNA (control)	RID ⁺ cows (n) with inserted BLV DNA	
		without high leukocytosis	with max leukocytosis
№ 1, <i>NK-lysin</i>	64.30±18.74 (n = 7)	54.49±8.98 (n = 16)	36.15±11.04 (n = 6)
№ 2, <i>NK-lysin</i>	27.43±9.17 (n = 10)	9.17±4.09 (n = 7)	3.76±1.22 (n = 8)
№ 1, <i>blvr</i>	7.10±1.50 (n = 7)	11.26±2.17 (n = 15)	12.18±2.37 (n = 6)
№ 1, <i>ifn-α</i>	40.90±21.67 (n = 10)	68.42±48.26 (n = 11)	1.90±0.84 (n = 6)
№ 2, <i>ifn-α</i>	—	—	21.80±12.00 (n = 7)

Note. BLV — bovine leukemia virus, RID⁻ and RID⁺ — negative and positive reaction of immunodiffusion, n — number of animals tested. Gene expression was normalized using ribosomal protein RPLPO reference gene. Gaps mean the absence of data.

Note that in both subgroups of RID⁺ cows without the insertion of BLV proviral DNA (BLV⁻) from two farms, there was an increment in the characteristics associated with innate immunity, compared to those recorded in animals carrying BLV proviral DNA. Thus, in the farm № 1 in RID⁺BLV⁻ cows, the blood neutrophil count reached $(3.59 \pm 0.61) \times 10^9/l$, whereas in animals with high leukocytosis was $1.66 \pm 0.35) \times 10^9/l$. Also, in the farm № 2 in RID⁺BLV⁻ cows, the *NK-lysin* expression was 13.62 ± 4.03 compared to 9.17 ± 4.09 for animals with moderate leukocytosis and 3.76 ± 1.22 for animals with the maximum leukocytosis (see Table 2). Apparently, RID⁺BLV⁻ cows in these two farms can have relatively increased activity of innate immunity and, accordingly, are able to eliminate BLV-infected cell clones more successfully.

Expression of the *blvr* gene in the farm from the Moscow region was higher in infected cows (see Table 2), but that did not correlate with an increase in the lymphocyte population. It was reported that *blvr* expression is detected in young B lymphocytes and decreases as they age [8]. Our results suggest a relatively increased contribution of young B lymphocytes to the lymphocyte cell population in animals infected by BLV.

Ifn-α expression was characterized by high individual variability (see Table 2). Apparently, this is due to the involvement of the *ifn-α* gene product in a wide range of signaling pathways and network interactions [15]. Nevertheless, in both farms, a tendency of decreased expression of *ifn-α* was observed in cows with high leukocytosis, compared to those free from BLV infection and with

moderate leukocytosis (see Table 2). In RID^+BLV^- animals, a tendency towards a relatively increased *ifn- α* expression (155.26 ± 106.17) appeared in the farm from the Penza region, which may also support the assumption of a relatively increased activity of natural antiviral protection systems in these animals.

In general, the obtained data suggests that, regardless of the severity of leukocytosis in BLV-infected animals, a decrease in *NK-lysin* expression, an increase in the population of young B lymphocytes (as the growth of *blvr* gene expression shows) and a certain tendency to a decline in interferon alpha gene (*ifn- α*) expression are detected in pronounced leukocytosis. It seems that the most reliable indicator of weakening protection against BLV, irrespectively of the individual features of peripheral blood ratio of granulo- and agranulocytes, can be a significant restriction of *NK-lysin* expression in BLV-infected animals, as identified by us in both farms (see Table 2). Interestingly, in our earlier studies, the sequencing of DNA fragments flanked by inverted repeat of (GAG)₆C microsatellite showed that in BLV-infected animals, unlike those free from the infection, this sequence is integrated into a structural gene encoding NK-lysine [16]. However, at this moment, there is no way to differentiate the presence of mutations that reduce the *NK-lysin* expression in BLV-infected cows from limiting the expression or reducing the proliferation of competent cells due to functional changes in the status of the immune system.

It is known that the initial stages of any infectious process are associated with activation of innate immune system and close network interactions between agranulocytes and granulocytes, whereas the final result of such interactions critically depends on the initial state of populations of white blood cells, the leukocytes [17]. It is likely that it is the insufficient knowledge of these relationships that causes the lack of success in the development of vaccination methods against such delta-retroviruses as BLV and T-cell lymphotropic virus type 1 (HTLV-1) [18].

The populations of regulatory T-cells (Treg) [19] are the leading factor of immunological homeostasis. Treg cells interfere with the activation of effector T lymphocytes involved in autoimmune and allergic processes, and, at the same time, reduce antitumor immunity. It was found that in cattle infected by BLV, activation of Treg cells is detected, which is accompanied, in particular, by a decrease in the cytotoxic activity of natural killers (NK-cells) [20, 21]. The proportion of Treg cells positively correlates with the number of lymphocytes, the virus titer and viral load in BLV infected cattle. There is experimental data showing that suppression of antiviral activity and cytotoxicity of NK-cells is due to activation of the secretion of growth-transforming beta factor (TGF- β) by Treg cells [22]. It should be emphasized that one of the effects of BLV proviral gene *tax* on host genes is an increase in the expression of tumor necrosis factor TNF- α [23, 24], which, in turn, activates Treg cells [25, 26]. In other words, the expression of BLV proviral DNA is accompanied by activation of TNF- α expression in the host. This induces Treg cells, the activation marker of which is the expression of TGF- β , which suppresses the proliferation and expression of cytotoxins in NK-cells, that corresponds to our data about the restriction of *NK-lysin* expression in BLV-infected cows in both farms.

The functional activity of NK cells directly depends on neutrophils, forming the axis of interaction between neutrophils and NK cells [27-29]. In recent years, accumulated experimental data showed that neutrophils, as an important source of cytokines and chemokines that activate other cells of the immune system, play a special role in regulating interactions in leukocyte populations during adaptive and innate immune response [18]. At the same time, the dependence of survival of neutrophils on interaction with platelets is reported [30]. Platelets,

among other properties, have a blocking effect on apoptosis [31].

The effect of BLV proviral DNA transcription resulted in a decrease in activity of T killers and NK cells and an increased population of platelets, the apoptosis blockers: BLV *tax* — activator of BLV proviral DNA transcription which influences the expression of a number of host genes, in particular TNF- α gene; TNF- α — tumor necrosis factor α , produced by lymphocytes; TGF- β — growth transformation factor β ; Treg — thymus-dependent population of regulatory cells.

The accumulated data allow us to propose a scheme for the effects of transcription of proviral DNA BLV after its insertion into the host genome: the transcriptional activator of the BLV proviral DNA Tax induces an increase in the amount of TNF- α in the host, which activates Treg cells that suppress, in particular, the cytotoxic function of NK cells (Fig.). This scheme corresponds to the experimental results obtained by us and gives grounds to assume that the key factors for prognosis of BLV retrovirus resistance, as well as possible targets in developing methods for its enhancement, lay in the metabolic pathways associated with activation of mechanisms of innate immunity and, in particular, NK lysin expression.

So, this study allows us to make the following conclusion. The number of peripheral blood erythrocytes in cows of different origins and from different farms may not be the same, but negative correlation of these differences with an average erythrocyte volume is statistically significant. In animals from different farms, the blood neutrophil level can differ substantially, which is accompanied by corresponding differences in the expression of the effector factor of innate immunity, the NK-lysin (i.e. the less neutrophils, the lower the expression of *NK-lysin* genes). Despite pronounced differences, in both surveyed farms the BLV infection of cows is realized against the background of a decreased NK-lysin expression compared to that of animals free from infection. One of the biomarkers of inflammatory processes is the increase in the counts of platelets, and according to this indicator, cows from different farms can also vary significantly. However, in animals infected by BLV, in comparison to those free from infection, a significant increase in the population of platelets exhibiting an anti-apoptotic function is detected, that, apparently, can provoke neoplastic transformation of cells.

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