Acta virologica **64**: 451 – 456, 2020 doi: 10.4149/av_2020_409

IFN-γ mRNA expression is lower in Holstein cows infected with bovine leukemia virus with high proviral load and persistent lymphocytosis

C. ÚSUGA-MONROY[1], L. G. GONZÁLEZ HERRERA[1], J. J. ECHEVERRI ZULUAGA[1], F. J. DÍAZ[2], A. LÓPEZ-HERRERA[1]

[1]Universidad Nacional de Colombia, Medellín campus, Faculty of Agricultural Sciences, Biodiversity and Molecular Genetics Group, Street 65 No. 59A-110, Medellín, Colombia; [2]Universidad de Antioquia, Faculty of Medicine, Immunovirology Group, Medellín, Colombia

Received November 19, 2019; revised May 1, 2020; accepted October 27, 2020

Summary. – Bovine leukemia virus (BLV) is a retrovirus that affects primarily milky cows. Animals serologically positive to BLV show a Th1 cytokine profile with a predominance of interferon gamma (IFN-γ). IFN-γ has antiviral activity through mechanisms such as resistance to infection, inhibition of viral replication and apoptosis. The objective of this work was to determine the transcription levels of IFN-γ and its relationship with proviral load and persistent lymphocytosis in a population of Holstein cows of the province of Antioquia, Colombia. IFN-γ transcription levels were evaluated by qPCR in 140 Holstein cows. A one-way analysis of variance and a Student's t test were used to evaluate the differences between the means. The amount of IFN-γ mRNA found in BLV-positive cows was lower than in BLV-negative cows. Moreover, in the group of infected cows a lower level of IFN-γ mRNA expression was found in BLV and persistent lymphocytosis cows (BLV+PL) compared with BLV and aleukemia cows (BLV+AL). The level of IFN-γ mRNA expression was lower in cows with high proviral load (HPL) compared to cows with low proviral load (LPL). BLV infection is related to abnormal expression of IFN-γ mRNA, although IFN-γ has antiviral activity, its expression is affected by high proviral load.

Keywords: cytokine; immune system; leukemia; bovine leukemia virus

Introduction

Bovine leukemia virus (BLV) is a retrovirus with ssRNA genome (Baltimore, 1971; Wu *et al*., 2003) that naturally infects cattle, buffaloes and capybaras. There is no commercial vaccine against BLV infection and the experimental vaccines do not elicit significant stimulation of bovine immune system (Gutiérrez *et al*., 2014). During the primary infection, the BLV actively replicates in the subset of CD5+ B cells and generates new free viral particles. In cattle not infected by BLV, 31% of B cells express CD5+ molecules; however, in BLV-infected cattle and in cattle with persistent lymphocytosis (PL), 90% of their B cells express CD5+ molecules (Stone *et al*., 1995). Infected B cells have an abnormal expression of cytokines and receptors caused by viral factors like Tax protein or microRNAs, losing reactivity to the presented antigens and allowing BLV replication (Frie and Coussens, 2015).

The cytokine profile of animals infected with BLV is related to the phase of the disease (Clerici and Shearer, 1993). Animals that are serologically positive to BLV without PL show a Th1 cytokine profile with a predominance of interferon gamma (IFN-γ), while animals with PL have a Th2 cytokine profile with a predominance of interleukin 10 (IL10) (Pyeon *et al*., 1996). Th1 cells produce more IFN-γ and induce the cell-mediated immune response and the neutralizing antibody response. IFN-γ is secreted by CD4+, CD8+, natural killer cells (NK), B cells, Natural killer T cells (NTK) and professional antigen presenting cells (APC;

E-mail: cusugam@unal.edu.co; phone: +574-440-90-55.

Abbreviations: AL = aleukemia; APC = antigen presenting cells; BLV = bovine leukemia virus; HPL = high proviral load; LPL = low proviral load; NK = natural killer cells; PL = persistent lymphocytosis

macrophages and dendritic cells) (Carnaud et *al.*, 1999; Harris et *al.*, 2000; Frucht et *al.*, 2001). This cytokine promotes macrophage activation, facilitates the presentation of antigens, regulates the Th1/Th2 balance and is involved in immunity against bacteria and viruses (Boehm et *al.*, 1997). The antiviral activity of IFN-γ occurs through various mechanisms (e.g. resistance to infection (Dhawan et *al.*, 1994), inhibition of viral replication and cellular apoptosis) and induces the Th1 type T cell response associated with antiviral protection (Ramshaw et *al.*, 1997). The role of IFN-γ in BLV infection has not been studied; however, the level of IFN-γ mRNA is higher in cattle with low proviral load (<100 copies/reaction) (Farias et *al.*, 2016). The objective of this work was to determine the transcription levels of the IFN-γ cytokine and its relationship with proviral load and with persistent lymphocytosis in a population of Holstein cows in the province of Antioquia.

Materials and Methods

Sample collection. One hundred and forty Holstein cows were evaluated between first and fifth lactation period and 3- to 7-year-old. The selected cows belonged to three dairy herds located in the municipalities of Medellín and Belmira (Antioquia) under the intensive specialized dairy system. Sampling was carried out during the months of February to June 2017. For the sampling, approval was obtained by the ethical committee of the Universidad Nacional de Colombia Medellín campus (CEMED-022, July 13, 2015). Two samples of blood were taken per animal with a time interval of three months; in each sample, two tubes (4 ml) of blood were collected in heparinized syringes by coccygeal venipuncture. The samples were homogenized by inversion and kept at 4°C.

DNA extraction from PBMC. Blood samples were transferred to 15 ml tubes and centrifuged for 4 min at 3,000 rpm at 4°C. The *salting out* technique was used to obtain the DNA from the buffy coat (Miller et *al.*, 1988). DNA was resuspended in TE buffer (1 M Tris HCl, 0.5 M EDTA, pH 8.0) (see below) and stored at 4°C until analysis. The quality and quantity of the DNA was evaluated on a spectrophotometer (NanoDrop2000) and on 1% agarose gel.

RNA extraction from PBMC and cDNA. One milliliter of blood was transferred to 15 ml conical tube and centrifuged for 15 min at 3,500 rpm at 4°C. One hundred microliters of the white layer containing the PBMC were transferred to a previously cooled 1.5 ml tube (4°C) with 2 µl of Ribolock RNAse Inhibitor (Thermo Scientific) and 1 ml of TRIzol Reagent (Life technologies). The total RNA extraction was performed according to the manufacturer's instructions using TRIzol Reagent. The quality and quantity of the total RNA obtained was verified through a spectrophotometer (NanoDrop2000). To remove the DNA present in the RNA sample, 16 µl of RNA was treated with 1 U of the enzyme DNaseI (ThermoScientific) at 37°C for 30 min. The reverse transcription reaction was carried out with 400 ng of DNase-treated RNA from each sample. The cDNA reaction was performed according to the manufacturer's instructions using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems). The quality of the cDNA obtained was confirmed through conventional PCR amplification of exon TLR6 (Arismendy-Morales *et al.*, 2017) and the reaction product was visualized on a 2% agarose gel in a gel documentator (Biorad).

BLV molecular detection (PCR). To determine the BLV infection in the Holstein cows, a nested PCR was performed for all samples. A region of the viral *env* gene was amplified to obtain a 444 bp fragment using the previously reported oligonucleotides (Beier *et al.*, 2001). The reaction was performed according to the protocol described by Úsuga-Monroy *et al.* (2015).

Peripheral blood smear. To determine persistent lymphocytosis, two samples were collected per cow with a time interval of three months between each sampling. For the peripheral blood smear, the wedge smear technique and the Wright stain was used. The slides were observed in an optical microscope at a magnification of 400x. The white cell population was counted in 100 visual fields. The number of white cells per microliter (µl) of peripheral blood was determined for each sample per animal. For the classification of persistent lymphocytosis (PL) status in bovines, if the two counts were greater than 10,000 white cells/µl of peripheral blood, the sample was classified as PL. If the two counts were less than 10,000 white cells/µl of peripheral blood, the sample was classified as aleukemia (AL) (Tajima *et al.*, 2003; Konnai *et al.*, 2005).

Proviral load. The qPCR reaction was performed in a final volume of 20 µl with 50 ng of DNA, 12.5 µl of Quanti Tect-SYBR (Qiagen) and 0.75 µl of 10 mM each oligonucleotide BLV-LTR 256 (5'-GAGCTCTCTTGCTCCCGAGAC-3') and BLV-LTR 453 (5'-GAAACAAACGCGGGTGCAAGCCAG-3') (Konnai et *al.*, 2005). The qPCR reaction was performed in a Rotor Gene thermocycler (Qiagen) under the following conditions: 15 s at 95°C, 40 cycles of 15 s at 94°C, 30 s at 58°C, and 30 s at 72°C. A negative control was included in each run and each sample was evaluated in duplicate. The standard curve was determined by serial dilution method (Jimba *et al.*, 2010). The number of copies/ng of DNA from a BLV positive sample was determined by serial dilution of the sample 15 times in 1xTE (1 mM Tris HCl, pH 7.5, 0.1 mM EDTA pH 8.0). The number of copies of the target gene (LTR) was determined according to the Poisson distribution model: $-\text{Log}_e f(x = 0)$, where f is the frequency of success (Jimba et *al.*, 2010; Hernández et *al.*, 2014). BLV-positive animals were classified as high proviral load (HPL), if they had more than 1,000 copies per reaction, or low proviral load (LPL), if they had less than 1,000 copies per reaction (Farias *et al.*, 2016).

IFN-γ mRNA expression level. The qPCR reaction was performed in a final volume of 20 µl with 40 ng of cDNA, 12.5 µl of Quanti Tect-SYBR (Quiagen) and 0.75 µl of 10 mM of each oli-

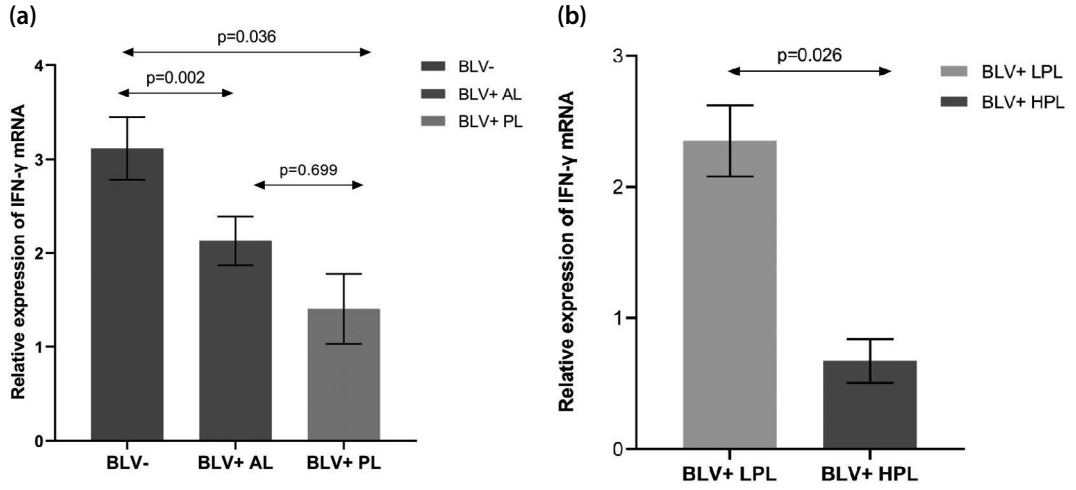

Fig. 1

Relative expression level of IFN-γ mRNA in PBMC obtained from BLV-negative cows and BLV-positive cows with AL and PL (a) and obtained from BLV-negative cows and BLV-positive cows with LPL and HPL (b)
The bars show the mean and standard error of the mean for each group. The p-value is presented on the bars in the graph. Statistical significance ($p < 0.05$).

gonucleotide IFN-γ-F (5'-CAGCTCTGAGAAACTGGAGGACTT-3') and IFN-γ-R (5'-TGGCTTTGCGCTGGATCT-3'). The β-actin gene was used as a reaction control in each sample, the primers for amplification were β-actin-F (5'-CGCACCACTGGCATTGTCAT-3') and β-actin-R (5'-TCCAAGGCGACGTAGCAGAG-3') (Konnai *et al.*, 2005; Farias *et al.*, 2016). The qPCR reaction was performed in a Rotor Gene thermocycler (Qiagen) under the following conditions: 15 s at 95°C, 40 cycles of 15 s at 94°C, 30 s at 58°C, and 30 s at 72°C. A negative control was included in each run; the IFN-γ gene and the β-actin gene were evaluated in duplicate in all cases.

Statistical analysis. The relative expression analysis of IFN-γ mRNA was performed using the $2^{-\Delta\Delta CT}$ method. A complete factorial experimental design was made. The data obtained were normalized by applying the square root transformation. A one-way analysis of variance and Student's t test was applied to evaluate the differences between the means of relative expression level of IFN-γ mRNA in BLV-negative cows and BLV-positive cows with AL and PL. The same analysis was used to evaluate the differences between the means of relative expression level of IFN-γ mRNA in BLV-positive cows with LPL or HPL. Pearson's correlation was used to determine the relationship between the relative expression of IFN-γ mRNA in BLV-positive cows with the number of total lymphocytes and the proviral load. The statistical significance of the differences ($p < 0.05$) was considered between groups. All statistical analyzes were carried out in the Gradpad Prism® V8 program (San Diego, United States) and in the SAS® version 9.2 program for Windows (SAS Institute Inc, Cary NC, USA).

Results

The molecular prevalence of BLV was 39.2% (55/140) in the Holstein cows. Sixty seven percent of BLV-positive cows had LPL and 32.7% of BLV-positive cows had HPL. Furthermore, 70.9% of BLV-positive cows were AL and 29.1% were classified as PL. BLV-positive cows had lower levels of IFN-γ cytokine expression compared to BLV-negative cows ($p = 0.0036$). Table 1 presents the data for the mean of the relative expression of IFN-γ mRNA in each of the groups evaluated. BLV-negative cows expressed more IFN-γ mRNA (3.375) compared to AL BLV-positive cows (2.129) and PL BLV-positive cows (1.401).

The mean IFN-γ mRNA expression level was significantly higher in negative cows compared to BLV+AL ($p = 0.002$) and BLV+PL cows ($p = 0.036$) (Fig. 1a). Relative expression of IFN-γ was higher in BLV+AL cows compared to BLV+PL cows, but these values were not statistically significant ($p = 0.699$).

Table 1. Relative expression of IFN-γ mRNA in PBMC obtained from BLV-negative cows, BLV-positive cows with aleukemia (AL) and BLV-positive cows with persistent lymphocytosis (PL)

Group	BLV-	AL	PL
Mean	3.375	2.129	1.401
Standard deviation	2.436	1.440	1.394
Standard error of the mean	0.3481	0.2586	0.3727

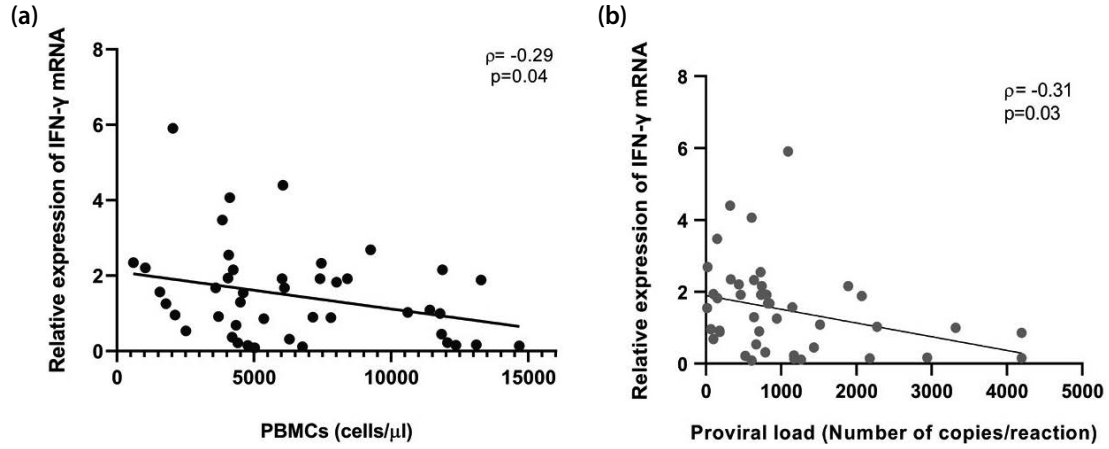

Fig. 2

Pearson's correlation between the relative expression of IFN-γ mRNA in PBMC obtained from cows positive to BLV and the number of total lymphocytes (a) and proviral load (b)

The p-value is presented on the graph for each of the groups. γ = Pearson's correlation coefficient. Statistical significance (p <0.05).

Figure 1b shows the relative expression of IFN-γ in BLV+LPL and BLV+HPL cows. The mean IFN-γ level was 1.68 times higher in LPL cows compared to HPL cows (p = 0.026).

The relative expression of IFN-γ mRNA of cows infected with BLV was negatively correlated with the amount of PBMC/μl (Fig. 2a) and with proviral load (Fig. 2b), although the correlation coefficients were relatively low (p = -0.31 and p = -0.36 for amount of PBMC and proviral load, respectively) both correlation coefficients were statistically significant p <0.05.

Discussion

Interferon has been associated with antiviral activity against viruses and is known to inhibit virus propagation and infection. The antiviral properties of IFN-γ have been demonstrated in *in vitro* models by inhibiting the replication of some retroviruses, including human T-cell lymphotropic virus (HTLV) (D'Onofrio *et al.,* 1992). In addition, IFN-γ reduces the number of syncytia formed by BLV (less than 1/20 in bovine fetal cells) when using recombinant bovine IFN-γ (rbIFN-γ) (Sentsui *et al.,* 2001).

Our analysis did not include the evaluation of other viral pathogens; it is possible that other secondary viral infections cause changes in IFN-γ mRNA expression in the cows. However, BLV-positive cows had lower of IFN-γ mRNA expression levels compared with BLV-negative cows. It is known that different stages of BLV infection (primary infection, persistent lymphocytosis, tumor stage) can modulate cytokine levels (Clerici and Shearer, 1993; Gutiérrez *et al.*, 2014). The IFN-γ production is mediated by CD4+ helper cells, CD8+ cytotoxic cells, NK and professional APCs (Carnaud *et al.*, 1999; Harris *et al.*, 2000; Frucht *et al.*, 2001). During adaptive immune response, the greatest amount of IFN-γ comes from T lymphocytes; it is therefore possible that BLV infection will down-regulate IFN-γ mRNA expression in T cells. The concentration of IFN-γ and IL-12 cytokines (pg/ml) in the supernatant of dendritic cell cultures from bovine infected with BLV has been shown to be lower compared to dendritic cells of the control group or BLV negative animals (Iwan *et al.*, 2018). The cellular immune response of CD4+ T lymphocytes is mediated by differentiation in the Th1 and Th2 subpopulations; both IFN-γ and IL-12 are the main inducers of Th1 lymphocytes. The immune response against BLV is conditioned and depends on the expression level of IFN-γ mRNA and the level of the IFN-γ protein in the cellular tissue. Our results show that BLV-infected bovines have lower IFN-γ mRNA levels, which may lead to macrophage activation, local inflammation or antiviral response not being effective in this group of animals and promoting viral replication.

In addition, our results show a lower level of IFN-γ mRNA expression in PL cows compared to AL cows. The development of PL in BLV+ cows is promoted by viral factors like the Tax protein or microRNAs (Szynal *et al.*, 2003; Tajima *et al.*, 2003; Gillet *et al.*, 2007). It is possible that BLV controls the host immune response by modifying the cytokine profile in infected animals, inhibiting apoptotic processes, and promoting infection. The results presented here determined a lower proportion of cows with LP (14/45), while 31 cows were classified as AL.

Although both groups of cows (AL and PL) were infected with BLV, it is possible that the antiviral activity of IFN-γ in BLV+AL cows may regulate polyclonal expansion of B cells and maintain the state of aleukemia. The antiviral activity of IFN-γ has been shown to be effective against BLV replication in effector cells and against the synthesis or integration of proviral DNA in B cells (Sentsui *et al.,* 2001).

The expression level of IFN-γ mRNA and the period of time for which this cytokine is excreted is important to control the viral pathogenesis of BLV. Cows infected with BLV with high levels of IFN-γ (for a period of up to 24 weeks after infection) did not develop LP, however cattle with high levels of IFN-γ for a shorter period of time after infection (3 at 4 weeks) entered PL (Yakobson *et al.,* 2000). It is possible that the cows infected with BLV with AL maintained the condition of aleukemia by having higher levels of IFN-γ expression for a longer period of time (Yakobson *et al.,* 2000) and thus have not developed PL.

Usui *et al.* (2007) reported lower levels of IFN-γ mRNA in sheep experimentally infected with BLV and showing high proviral load compared to sheep with low proviral load. Similarly, Farias *et al.* (2016) analogous changes in the levels of IFN-γ in PBMC of cows naturally infected with BLV. These results were similar to those found here, where BLV-positive cows with LPL showed higher expression of IFN-γ mRNA compared to BLV-positive cows with HPL ($p = 0.0001$).

The relative expression of IFN-γ mRNA from infected cows was negatively correlated with the number of PBMC cells/μl and with proviral load. The ability of NK cells to produce IFN-γ is inversely correlated with the proviral load of BLV, in addition, the cytotoxicity of NK cells is lower in BLV-positive cows with PL (Ohira *et al.,* 2016). This is similar with the results presented in figure 3B, where the higher proviral load was, the lower was the IFN-γ mRNA level ($p < 0.05$).

The lower production of IFN-γ has been correlated with low expression of perforins in cows with PL, which reduces the cytotoxicity of NK during infection; however, the cytotoxic ability of NKs improves in the presence of IFN-γ to eliminate tumors and infected cells by releasing pro-inflammatory granules and cytokines (Ohira *et al.,* 2016). Increased level of IFN-γ expression stimulates cell immunity, promotes cytotoxic cell polarization, activates macrophages, and induces maturation of APCs (Stephens *et al.,* 2003). IFN-γ induces the trans-activator of the major class II histocompatibility complex (CIITA) through the JAK-STAT pathway, thus inhibiting viral replication at the transcriptional level, as in cells latently infected with HIV (Sarol *et al.,* 2002).

IFN-γ can control the viral infection caused by BLV by maintaining the state of aleukemia in positive cows; however, the high proviral load induces the reduction of IFN-γ mRNA. The role of IFN-γ during the BLV infection process is important, its relationship with other cytokines and viral factors must be analyzed evaluate its antiviral potential.

Acknowledgments. This project was funded by the Research Direction of the Universidad Nacional de Colombia, Medellín Campus. Project HERMES 44328. We want to thank the producers that were involved in the development of the project.

References

Arismendy-Morales J, López-Herrera A, Echeverri J (2017): Association of TLR6 gene polymorphisms with zootechnical parameters in Holstein cattle in northern Antioquia. Rev. Fac. Nac. Agron. 70, 8203–8211. https://doi.org/10.15446/rfna.v70n2.60766

Baltimore D (1971): Expression of Animal Virus Genomes. Bacteriol. Rev. 35, 235. https://doi.org/10.1128/MMBR.35.3.235-241.1971

Beier D, Blankenstain P, Marquard O, Kuzmak J (2001): Identification of different BLV provirus isolates by PCR-RFLP and DNA sequencing. Berl Munch Tierarztl Wochenschr. 114, 252–256.

Boehm U, Klamp T, Groot M, Howard J (1997): Cellular responses to interferon-gamma. Annu. Rev. Immunol. 15, 749–795. https://doi.org/10.1146/annurev.immunol.15.1.749

Carnaud C, Donnars O, Park S, Beavis A, Koezuka Y, Bendelac A (1999): Cutting edge: cross-talk between cells of the innate immune system: NKT cells rapidly activate NK cells. J. Immunol. 163, 4647–4650.

Clerici M, Shearer, M (1993): A TH1>TH2 switch is a critical step in the etiology of HIV infection. Immunol. Today 14, 107–111. https://doi.org/10.1016/0167-5699(93)90208-3

D'Onofrio C, Franzese O, Puglianiello A, Peci E, Lanzilli G, Bonmassar E (1992): Antiviral activity of individual versus combined treatments with interferon alpha, beta and gamma on early infection with HTLV-I in vitro. Int. J. Immunopharmacol. 14, 1069–1079. https://doi.org/10.1016/0192-0561(92)90152-B

Dhawan S, Heredia A, Lal B, Wahl M, Epstein J, Hewlett I (1994): Interferon-gamma induces resistance in primary monocytes against human immunodeficiency virus type-1 infection. Biochem. Biophys. Res. Commun. 201, 756–761. https://doi.org/10.1006/bbrc.1994.1765

Farias MVN, Lendez PA, Marin M, Quintana S, Martínez-Cuesta L, Ceriani MC, Dolcini GL (2016): Toll-like receptors, IFN-γ and IL-12 expression in bovine leukemia virus-infected animals with low or high proviral load. Res. Vet. Sci. 107, 190–195. https://doi.org/10.1016/j.rvsc.2016.06.016

Frie M, Coussens P (2015): Bovine leukemia virus: A major silent threat to proper immune responses in cattle. Vet. Immunol. Immunopathol. 163, 103–114. https://doi.org/10.1016/j.vetimm.2014.11.014

Frucht D, Fukao T, Bogdan C, Schindler H, O'Shea J, Koyasu S (2001): IFN-gamma production by antigen-presenting cells: mechanisms emerge. Trends Immunol. 22, 556–560. https://doi.org/10.1016/S1471-4906(01)02005-1

Gillet N, Florins A, Boxus M, Burteau C, Nigro A, Vandermeers F, Willems L (2007): Mechanisms of leukemogenesis induced by bovine leukemia virus: prospects for novel anti-retroviral therapies in human. Retrovirology 4, 1–32. https://doi.org/10.1186/1742-4690-4-18

Gutiérrez G, Rodríguez S, Brogniez A, Gillet N, Golime R, Burny A, Willems L (2014): Vaccination against – Retroviruses: The Bovine Leukemia Virus Paradigm. Viruses 6, 2416–2427. https://doi.org/10.3390/v6062416

Harris D, Haynes L, Sayles P, Duso D, Eaton S, Lepak N, Lund F (2000): Reciprocal regulation of polarized cytokine production by effector B and T cells. Nat. Immunol. 1, 475–482. https://doi.org/10.1038/82717

Hernández D, Muñoz J, Alvarez L (2014): Association between the locus BOLA-DRB3.2 and bovine leukemia virus in creole colombian breeds. Rev. Colombiana. Cienc. Anim. 6, 319–326. https://doi.org/10.24188/recia.v6.n2.2014.435

Iwan E, Szczotka M, Kocki J, Pluta A (2018): Determination of cytokine profiles in populations of dendritic cells from cattle infected with bovine leukaemia virus. Pol. J. Vet. Sci. 21, 681–690.

Jimba M, Takeshima S, Matoba K, Endoh D, Aida Y (2010): BLV-CoCoMo-qPCR: Quantitation of bovine leukemia virus proviral load using the cocomo algorithm. Retrovirology 7, 1–19. https://doi.org/10.1186/1742-4690-7-91

Konnai S, Usui T, Ikeda M, Kohara J, Hirata T, Okada K, Ohashi K, Onuma M (2005): Imbalance of tumor necrosis factor receptors during progression in bovine leukemia virus infection. Virology 339, 239–248. https://doi.org/10.1016/j.virol.2005.06.010

Miller S, Dykes D, Polesky H (1988): A simple salting out procedure for extracting ADN from human nucleated cells. Nucleic Acids Res. 16, 1215. https://doi.org/10.1093/nar/16.3.1215

Ohira K, Nakahara A, Konnai S, Okagawa T, Nishimori A, Maekawa N, Ikebuchi R, Kohara J, Murata S, Ohashi K (2016): Bovine leukemia virus reduces anti-viral cytokine activities and NK cytotoxicity by inducing TGF-β secretion from regulatory T cells. Immun. Inflamm. Dis. 4, 52–63. https://doi.org/10.1002/iid3.93

Pyeon D, O'Reilly K, Splitter G (1996): Increased interleukin-10 mRNA expression in tumor-bearing or persistently lymphocytotic animals infected with bovine leukemia virus. J. Virol. 70, 5706–5710. https://doi.org/10.1128/JVI.70.8.5706-5710.1996

Ramshaw I, Ramsay A, Karupiah G, Rolph M, Mahalingam S, Ruby C (1997): Cytokines and immunity to viral infections. Immunol. Rev. 159, 119–135. https://doi.org/10.1111/j.1600-065X.1997.tb01011.x

Sarol L, Imai K, Asamitsu K, Tetsuka T, Barzaga N, Okamoto T (2002): Inhibitory effects of IFN-y on HIV-1 replication in latently infected cells. Biochem. Biophys. Res. Commun. 291, 890–896. https://doi.org/10.1006/bbrc.2002.6532

Sentsui H, Murakami K, Inoshima Y, Yokoyama T, Inumaru S (2001): Anti-viral effect of recombinant bovine interferon y on bovine leukaemia virus. Cytokine 16, 227–231. https://doi.org/10.1006/cyto.2001.0967

Stephens S, Brownlie J, Charleston B, Howard C (2003): Differences in cytokine synthesis by the sub-populations of dendritic cells from afferent lymph. Immunology 110, 48–57. https://doi.org/10.1046/j.1365-2567.2003.01712.x

Stone D, Hof A, Davis W (1995): Up-regulation of IL-2 receptor alpha and MHC class II expression on lymphocyte subpopulations from bovine leukemia virus infected lymphocytotic cows. Vet. Immunol. Immunopathol. 48, 65–76. https://doi.org/10.1016/0165-2427(95)05423-4

Szynal M, Cleuter Y, Beskorwayne T, Bagnis C, Van Lint C, Kerkhofs P, Van den Broeke A (2003): Disruption of B-cell homeostatic control mediated by the BLV-Tax oncoprotein: association with the upregulation of Bcl-2 and signaling through NF-kappa B. Oncogene 22, 4531–4542. https://doi.org/10.1038/sj.onc.1206546

Tajima S, Takahashi M, Takeshima S, Konnai S, Ai Yin S, Watarai S, Aida Y (2003). A mutant form of the tax protein of bovine leukemia virus (BLV), with enhanced transactivation activity, increases expression and propagation of BLV in vitro but not in vivo. J. Virol. 77, 1894–1903. https://doi.org/10.1128/JVI.77.3.1894-1903.2003

Usui T, Konnai S, Ohashi K, Onuma M (2007): Interferon-gamma expression associated with suppression of bovine leukemia virus at the early phase of infection in sheep. Vet. Immunol. Immunopathol. 115, 17–23. https://doi.org/10.1016/j.vetimm.2006.09.011

Úsuga-Monroy C, Echeverri JJ, López-Herrera A (2015): Diagnóstico molecular del virus de leucosis bovina en una población de vacas Holstein, Colombia. Archivos de Zootecnia 64, 383–388. https://doi.org/10.21071/az.v64i248.424

Wu D, Murakami K, Morooka A, Jin H, Inoshim Y, Sentsui H (2003): In vivo transcription of bovine leukemia virus and bovine immunodeficiency-like virus. Virus Res. 97, 81–87. https://doi.org/10.1016/S0168-1702(03)00222-3

Yakobson B, Brenner J, Ungar-Waron H, Trainin Z (2000): Cellular immune response cytokine expression during the initial stage of bovine leukemia virus (BLV) infection determines the disease progression to persistent lymphocytosis. Comp. Immun. Microbiol. Infect. Dis. 23, 197–208. https://doi.org/10.1016/S0147-9571(99)00074-0