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Original article

# Cytokine profiles of dendritic cells (DCs) during infection with bovine leukaemia virus (BLV)

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## Abstract

BLV is an agent of enzootic bovine leukaemia (EBL), an infectious disease affecting cattle worldwide. BLV infection has been associated with immune system disorders and discrepancies in the cytokine network. The significance of dendritic cells in the pathogenesis of BLV infection is largely unknown, but considering their fundamental role in immune response it may be crucial. DCs precursors were isolated with the use of immunomagnetic beads from BLV-infected and BLV-free cows. From these precursors cultures of monocyte derived dendritic cells (MoDCs) were generated with the use of a cytokine cocktail (IL-4 and GM-CSF). Additionally, parallel DCs from BLV-negative animals were infected *in vitro*. The level of cytokines: IL-6, IL-10, IL-12(p40), IL-12(p70) was determined in DC cultures: infected *in vitro*, originating from naturally infected cattle and BLV-free cattle. The investigation showed significant changes in almost all analyzed populations of BLV-infected DCs. Cytokine profiles of blood MoDCs indicated activation of these groups during infection. In the case of spleen MoDCs and lymph node MoDCs a decrease in production of IL-12(p40) and IL-12(p70) in favour of IL-6 and IL-10 was noted, suggesting promotion of BLV infection development.

**Key words:** Bovine leukaemia virus (BLV), dendritic cells (DCs), cytokines.

## Introduction

Bovine leukaemia virus (BLV) belongs to the *Retroviridae* family, *Deltaretrovirus* genus and is closely related to the human T-cell leukaemia virus-1 (HTLV-1). It is an etiological agent of enzootic bovine leukaemia (EBL), a lymphoproliferative, infectious disease of cattle. Because of economic losses in the cattle industry worldwide, BLV infections are considered to be a significant issue. While strict regula-

tions and restrictions in most of Western Europe allowed it to obtain EBL-free status, in Poland this disease is still a concern.

Persistent BLV infection can result in an oncogenic process, evoking cell transformation and leukaemia. EBL can manifest in three stages: asymptomatic or aleukemic (AL), persistent lymphocytosis (PL), and leukaemia or lymphoma (Frie and Coussens 2015). Transmission may occur both vertically and horizontally, generally through blood, less often by se-

men or milk. The main target cells for BLV are B cells, but the virus also can infect other types of cells: T cells, monocytes, macrophages and dendritic cells (DCs) (Stott et al. 1991, Heeney et al. 1992, Domenech et al. 2000, Wu et al. 2003, Iwan et al. 2014).

DCs are constantly produced from hematopoietic stem cells (HSC) in bone marrow. They are the most potent antigen presenting cells (APCs), having the capacity to take up, process and present antigens to naïve T cells in lymphoid tissues (Palucka and Banchereau 2012). Present on the surface of DCs in large numbers are molecules vital for antigen presentation: major histocompatibility complexes (MHC) I and II class, CD40, CD80 and CD86. Through the expression of surface molecules and cytokine secretion, dendritic cells can initiate or suppress immunological reactions. Furthermore, by affecting the Th1/Th2 balance DCs are able to influence the efficiency of the immune response (Stephens et al. 2003). DCs are crucial for the antiviral response of the organism and provide an essential link between innate and adaptive immunity (Flint et al. 2009). They also induce immunological tolerance and take part in creating immune memory (Banchereau and Steinman 1998).

BLV may affect cells of the immune system on many levels, it can influence proliferation, apoptosis and expression of receptors and cytokines, which are especially important for immune defence (Kabeya et al. 2001, Frie and Coussens 2015). Cytokines are accountable for cell-to-cell communication; also, by affecting growth, differentiation and sensitivity of cells, they can regulate the strength and length of immunological reactions (Kabeya et al. 2001). Based on the type of immune response in which they partake, cytokines can be divided into two major categories: Th1, where cells secrete mostly: IL-2, IFN- $\gamma$ , IL-12, and Th2 – IL-4, IL-5, IL-6, IL-10 (Lucey et al. 1996). The cytokine microenvironment has been established as one of the major factors during antitumor and antiviral immunity. Disturbance in the regulation of the cytokine network plays an essential role in the pathogenesis of persistent viral infections and oncogenesis (Frie and Coussens 2015). Moreover, specific cytokine profiles have been correlated with cancer progression and used to predict disease prognosis (Lee and Margolin 2011).

Considering their key role in immunity, DCs are an attractive target for viral infection. Detailed investigation of cytokine secretion profiles can provide valuable information about how viral infection affects DCs and their relevance during BLV pathogenesis.

## Aim

The aim of the study was the determination of cytokine profiles in cultures of both naturally and *in vitro* BLV infected dendritic cells originating from different tissues.

## Materials and Methods

### Animals

The study was performed on samples collected from 20 polish black and white lowland breed cows: ten BLV-positive and ten BLV-negative. BLV infection was confirmed by ELISA (gp51 and p24 markers, IDEXX) and nested PCR (*env* gen). Additionally, hematological examinations of leukocyte levels and percentage compositions of white blood cells (Schilling formula) were performed, where all BLV-positive animals were classified as aleukemic. All samples in the study were collected during routine examinations or slaughter of cattle, thus ethical approval for animal experimentation was not required. Peripheral blood was taken from the jugular vein with EDTA/K<sub>2</sub> as anticoagulant. Samples from bone marrow, spleen and lymph nodes were collected directly after slaughter.

### Samples preparation

Blood was centrifuged 1 h at 1125 x g (Sigma 4K15, Germany) at 8° C and a pellet of leukocytes was collected. Fragments of spleen and lymph nodes were homogenized. Cells from blood, bone marrow, spleen and lymph nodes were suspended 1:1 in PBS buffer (pH-7.2, Sigma-Aldrich) and centrifuged in density gradient (Histopaque 1077; Sigma-Aldrich) for 45 min at 1000 x g at 8° C. Fractions of mononuclear cells were collected, rinsed in PBS buffer and counted.

### Isolation of dendritic cell precursors

Precursors (monocytes CD14+) were isolated from 1 x 10<sup>7</sup> mononuclear cells of blood, bone marrow, spleen and lymph nodes of both naturally infected and BLV-free animals by immunomagnetic separation. The cells were suspended in isolation buffer (PBS, pH – 7.2, 2 mM EDTA; Miltenyi Biotec with 0.5% calf serum; Sigma-Aldrich) and incubated for 15 min at 4° C with 20  $\mu$ L of CD14 MicroBeads (Miltenyi Biotec). After incubation, the cells were washed, centrifuged at 300 x g for 10 min. Fractions of CD14+

were collected during magnetic separations, performed on LS Columns (Miltenyi Biotec) according to the manufacturer's instruction.

### Generation and cultivation of monocyte derived dendritic cells (MoDCs)

Monocytes CD14+ were cultured in RPMI 1640 medium (Gibco) containing 20% calf serum (Sigma-Aldrich), 0.3 mg/mL of L-glutamine (Sigma-Aldrich), antibiotic-antimycotic solution (Sigma-Aldrich) in 1:100 dilution, GM-CSF (Bio-Rad/Serotec) in 1:500 dilution and 1 ng/mL of IL-4 (Endogen). The precursors were sustained in 10 mL culture flasks (Nunc), in an atmosphere of 5% CO<sub>2</sub> and at 37°C. Adherent cultures of monocyte derived dendritic cells (MoDCs) were formed after 5 days of stimulation with cytokine cocktail (IL-4 and GM-CSF). Generated cultures were cultivated as described above. MoDCs from BLV-free animals, naturally infected animals and DCs infected *in vitro* were maintained for two weeks at a concentration of 0.5 x 10<sup>6</sup> cell/mL. MoDC cultures from BLV-negative cattle were prepared in double, one set was used as control, the other was subjected to an additional step of *in vitro* infection with BLV.

### Cell line

Fetal lamb kidney cell line permanently infected with BLV (FLK-BLV) was used as a source of the virus.

### Infection of MoDCs *in vitro*

The medium of FLK/BLV cell line culture was collected and centrifuged at 2000 x g for 20 min. Cell free supernatant was used for MoDCs infection. 5 ml of FLK/BLV supernatant was added to adherent culture of MoDCs from BLV-negative animals and incubated for 5 h at 5% CO<sub>2</sub> and 37°C. The culture medium was then discarded, MoDCs were rinsed in PBS buffer (pH-7.2, Sigma-Aldrich), suspended in fresh medium and cultured as indicated above. BLV infection in DCs cultures was confirmed by nested PCR and *in situ* PCR (Iwan et al. 2014).

### Measurement of cytokine concentrations by ELISA

After two weeks 2 mL of medium was collected from MoDCs cultures (0.5 x 10<sup>6</sup> cells/mL) and centri-

fuged for 10 min at 1100 x g; cell-free supernatants were then collected, lyophilized and stored at -20°C until use. Directly before analysis lyophilized samples were suspended in 1 ml of double-distilled water. Levels of IL-6, IL-10, IL-12(p40) and IL-12(p70) in the samples were measured by ELISA OptEIA (BD), according to manufacturer instruction. Concentrations of cytokines were calculated based on standard curves. Results were given in pg/ml calculated for approximately 1 x 10<sup>6</sup> cells. Statistical analysis was performed using U Mann-Whitney with the use of Statistica 10 software, *p*-value was accepted at 0.05 level.

## Results

Concentrations of IL-6, IL-10 IL-12(p40) and IL-12(p70) were analyzed in supernatants from naturally and *in vitro* BLV infected cultures of MoDCs generated from blood, bone marrow, spleen and lymph nodes. BLV-negative MoDCs were used as control.

### Cytokine levels in MoDCs cultures of cattle naturally infected with BLV

In the supernatants of MoDCs generated from the blood of BLV-positive animals concentrations of IL-6 and IL-10 were higher than those observed in the control group. Statistical analysis showed that these differences were significant. The average concentration of IL-12(p40) in the examined group was increased and the difference was substantial – almost double the control value, but still not statistically significant. This may suggest a tendency to higher secretion of IL-12(p40) by MoDCs from the blood of naturally infected animals. Additionally, it was shown that the average level of IL-12(p70) was almost the same in cultures of both BLV-negative and BLV positive blood MoDCs (Table 1 and Fig. 1).

In cultures of MoDCs generated from the bone marrow of BLV-infected animals statistically significant differences in cytokine secretion were not observed. Nonetheless, in the experimental group a tendency to higher production of IL-10, IL-12(p40) and IL-12(p70), compared to the control group was noted. On the other hand, decrease in mean concentration of IL-6 in comparison to cultures originating from BLV-negative animals was shown (Table 1 and Fig. 2).

Table 1. Average interleukin concentration (pg/ml) in dendritic cells of: BLV positive animals (BLV+), MoDCs infected *in vitro* and BLV negative animals (BLV -).

Origin		IL-6	IL-10	IL-12 (p40)	IL-12 (p70)
<i>MoDCs</i>		<i>Average interleukin concentration in pg/ml</i>			
Blood	BLV +	26.62	4.39	32.29	3.04
	<i>in vitro</i> infected	4.10	4.18	12.25	2.70
	BLV -	17.32	3.33	15.07	3.00
Bone marrow	BLV +	15.59	4.07	14.90	3.10
	<i>in vitro</i> infected	3.06	2.47	10.37	2.50
	BLV -	17.78	3.06	12.81	2.86
Spleen	BLV +	6.40	7.03	19.30	3.53
	<i>in vitro</i> infected	2.04	2.54	9.80	2.30
	BLV -	3.12	3.50	22.15	3.90
Lymph nodes	BLV +	17.15	12.09	10.80	3.63
	<i>in vitro</i> infected	2.07	2.42	9.70	2.50
	BLV -	8.80	7.70	14.50	3.80

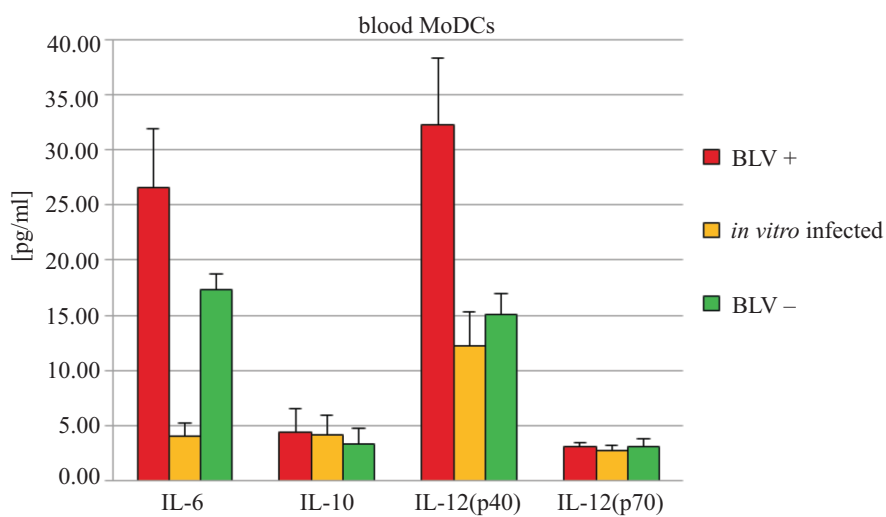


Fig. 1. Average concentration (pg/ml) of cytokines in cultures of blood MoDCs: BLV positive animals (BLV+), MoDCs infected *in vitro* and BLV negative animals (BLV-).

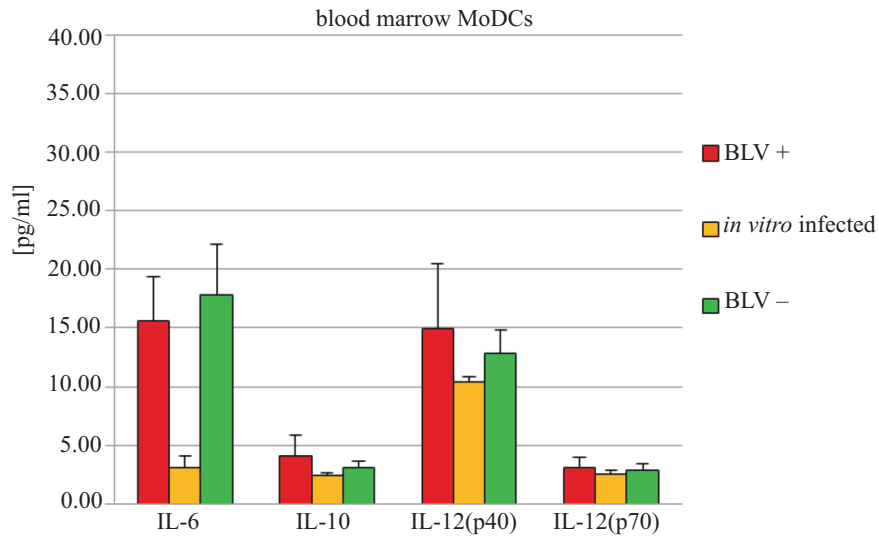


Fig. 2. Average concentration (pg/ml) of cytokines in cultures of bone marrow MoDCs: BLV positive animals (BLV+), MoDCs infected *in vitro* and BLV negative animals (BLV -).

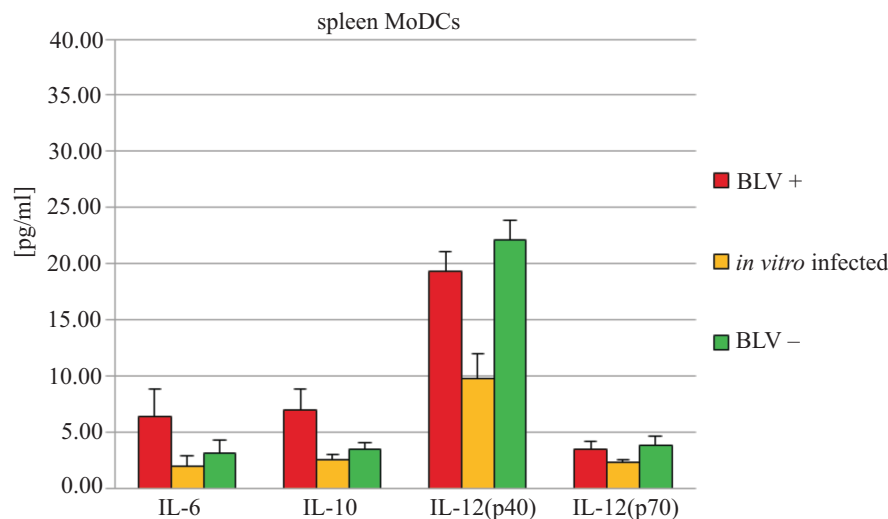


Fig. 3. Average concentration (pg/ml) of cytokines in cultures of spleen MoDCs: BLV positive animals (BLV+), MoDCs infected *in vitro* and BLV negative animals (BLV -).

The secretion profile of spleen MoDCs from naturally infected animals indicated a statistically significant elevation in IL-10 and IL-6 production. Average levels of these cytokines were twice as high as in BLV-free cultures. Additionally, in spleen MoDCs cultures originating from BLV-positive cattle, significant decreases in secretion of both IL-12 forms were observed (Table 1 and Fig. 3).

Similarly to the spleen MoDCs, MoDCs generated from the lymph nodes of naturally infected animals were characterized by increased secretion of IL-10 and IL-6 and decreased production of both forms of IL-12 (Table 1 and Fig. 4).

#### Cytokine levels in MoDCs cultures infected with BLV *in vitro*

In MoDCs infected *in vitro* secretion of almost all analyzed cytokines was decreased in comparison to BLV-free controls.

In cultures of blood MoDCs infected *in vitro* with BLV only in the case of IL-10 elevated secretion was noted. Additionally, in this cell population production of both IL-6 and IL-12 forms was lowered, compared to controls (Table 1 and Fig. 1).

In MoDCs from bone marrow infected *in vitro* the most significant decrease was shown in produc-

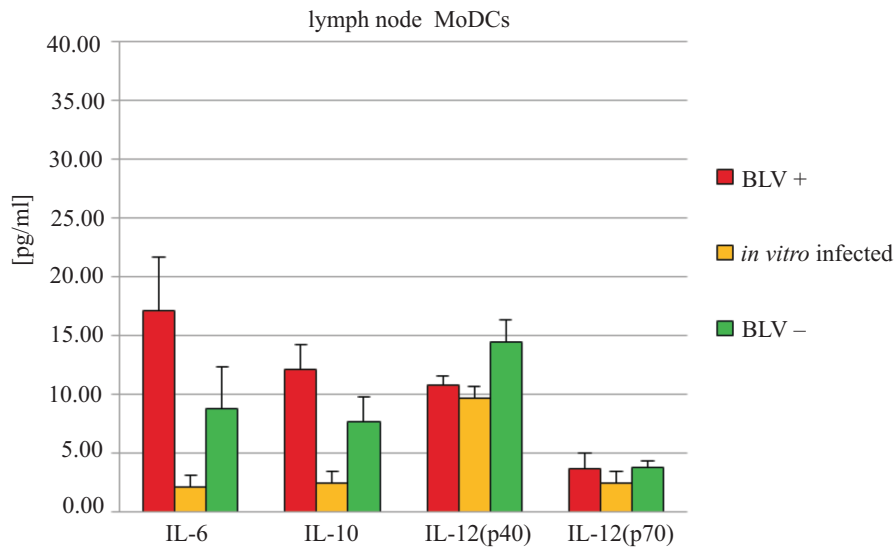


Fig. 4. Average concentration (pg/ml) of cytokines in cultures of lymph node MoDCs: BLV positive animals (BLV+), MoDCs infected *in vitro* and BLV negative animals (BLV -).

tion of IL-6. Its average concentration in this group was several times lower than the level of IL-6 in analogous BLV-free cultures. Average levels of the remaining examined interleukins were also lower than those observed in the control group (Table 1 and Fig. 2).

In spleen MoDCs infected *in vitro* with BLV a large decline in production of IL-12 (p40) was observed. The average concentration of IL-12(p40) in these cultures was decreased twofold compared to analogous BLV-negative MoDCs. Mean levels of: IL-6, IL-10 and IL-12(p70) in the examined group were also significantly lower in comparison to the controls (Table 1 and Fig. 3).

MoDCs generated from lymph nodes and infected *in vitro* were characterized by a significantly diminished secretion of IL-6 and IL-10. Average concentrations of both these cytokines were reduced threefold in comparison to the control groups. A less relevant decrease was observed in the case of both IL-12 forms (Table 1 and Fig. 4).

In addition it could be observed that the secretion profiles of all MoDCs after BLV infection *in vitro* were quite similar, regardless of the tissue from which they originated.

## Discussion

The issue of BLV infection has been widely described in regard to many types of immune cells, except dendritic cells. The role of DCs during BLV pathogenesis is still largely unknown and may be substantial, if other *Retroviridae*, e.g. HIV or HTLV are

any indicators. In the case of these viruses DCs are not only infected but can also actively partake in viral pathogenesis and virus propagation (Flint et al. 2009, Pique and Jones 2012).

Dendritic cells are a key element of body anti-viral response, which make them an attractive target for persistent viral infection (Flint et al. 2009). The cytokine profile of DCs depends mostly on the origin of analyzed cells, the microenvironment and outside factors, such as pathogens (Stephens et al. 2003). Detailed investigation of secretion profiles can provide valuable information about this cell population during BLV pathogenesis and how the virus affects DCs themselves.

In this study production of four cytokines: IL-6, IL-10, IL-12(p40) and IL-12(p70), was investigated in both naturally and *in vitro* BLV-infected DCs cultures. This *in vitro* study showed that there are distinct differences between the analyzed populations, depending on their origin and the nature of the BLV infection. Observed differences could significantly affect immune response *in vivo* and its outcome.

Additionally, it should be emphasised that for purpose of this study, as an accessible source of DCs, precursors were used. As a result, in the case of naturally infected animals cells were infected with BLV *in vivo*, in unknown conditions, as DC precursors, while in the case of *in vitro* infection with BLV, already formed DCs undergo the infection process in clearly specified conditions.

Interleukin-12 is a proinflammatory cytokine, produced by antigen presenting cells, mainly DCs. It is one of the most important cytokines in the polarisation of naive T cells to interferon producing

Th1 (Lucey et al. 1996, Yakobson et al. 1998, Pyeon and Splitter 1999). It has been shown that IL-12 can stimulate the expression of Th1 cytokines, actively counteracts apoptotic processes and that it has strong antitumoral and antiviral properties. IL-12 is a heterodimer (p70) composed of two subunits: p35 and p40, which in particular can be secreted independently as a monomer and still be biologically functional (Vignali and Kuchroo 2012). Expression of IL-12(p70) and IL-12(p40) can differ, especially in the case of DCs; this is why both forms of IL-12 were examined separately.

The results of the present study showed that production of IL-12(p40) monomer was significantly higher as compared to IL-12(p70) complex in all examined MoDCs populations, regardless of infection status. This seems to be consistent with the literature indicating that DCs do not constantly express the p35 subunit and as a result their secretion of the IL-12(p70) heterodimer compared to p40 may be modest (Abdi 2002, Stephens et al. 2003).

In the examined group of naturally infected MoDCs from blood the results indicated a higher secretion of IL-12(p40), while in the case of IL-12(p70) no significant differences were observed. Similar regularity was observed by Stephens et al (2003). They suggested that the elevated expression of IL-12(p40) in DCs cultures, rather than the whole IL-12(p70) complex, is an early host response to pathogen infection and that this may account for skewing of the T cell response in the direction of the Th1 cytokine pattern. While IL-12(p40) is a chemotactic cytokine with the ability to activate dendritic cells, it has also been shown that by competition for receptors with IL-12(p70) it can indirectly impair immune reaction (Méndez-Samperio 2010). Considering this aspect in naturally infected blood MoDCs, the significant increase of p40 production, while expression of the whole complex shows no change, may also suggest weakening of the immunologic response. Whether in this case the observed differences are due to less efficient and delayed expression of IL-12(p70) in DCs, or are actually related to BLV infection, needs further confirmation. Additionally, in blood MoDCs cultures from naturally infected, aleukemic cattle, the results seem to be consistent with those obtained from PBMCs (peripheral blood mononuclear cells) cultures of aleukemic animals, where increased expression of IL-12(p40) was also noted (Pyeon and Splitter 1998, Yakobson et al. 2000, Muller et al. 2003).

In the population of naturally infected MoDCs originating from bone marrow a slight elevation in concentrations of both IL-12 forms were noted, but the discrepancies were not statistically significant

and were probably related to tissue specificity and individual variabilities of studied samples.

The current study also showed a significant decline of IL-12(p40) and IL-12(p70) secretion in lymph node and spleen DCs originating from BLV-positive animals. This occurrence may suggest a more advanced state of BLV infection in these cell populations (Pyeon and Splitter 1998, Yakobson et al. 2000, Kabeya et al. 2001, Konnai et al. 2003, Muller et al. 2003). While it was established that aleukemic PBMCs express significantly more IL-12, analogous cultures from leukemic animals produce much less of this cytokine, in comparison to BLV-negative controls (Pyeon and Splitter 1998, Yakobson et al. 2000, Muller et al. 2003). Similar results were also observed by Usui et al. (2007) in PBMCs cultures of BLV infected sheep. Considering the crucial role of DCs and IL-12 during immune response to viral infection, suppression of IL-12 secretion in DCs could be very advantageous for the progress of BLV pathogenesis (Kabeya et al. 2001, Konnai et al. 2003, Frie and Coussens 2015).

In the case of MoDCs generated from all four analyzed tissues and infected *in vitro* with BLV, significant decreases in levels of both IL-12 forms were noted. This suggests drastic deterioration of IL-12 expression shortly after BLV *in vitro* infection, regardless of MoDCs origin. Such a substantial drop was not observed in analogous DCs cultures originating from naturally infected animals. The differences noted between those two DCs groups are probably due to several factors: the time that passed from primary infection, infectious dosage of the virus and DCs microenvironment. In MoDCs from naturally infected cattle the time of infection and viral dosage are impossible to determine. Furthermore, the *in vivo* environment is much more complex than culture *in vitro*, and these additional factors should be taken into consideration.

Interleukin-6 is a proinflammatory cytokine involved in the initiation of Th2 response; it also takes part in the activation of T cells and induction of B cell differentiation in plasmoblasts (Lee and Margolin 2011, Frie and Coussens 2015). However, it has also been shown that IL-6 can actively promote the growth of tumorous cells (Svenson et al. 2013, Landskron et al. 2014). IL-6, as one of the most often deregulated cytokines, is frequently used, as a prognostic marker for various cancers – abnormally elevated levels are associated with a poor prognosis (Szcotka et al. 2005, Lee and Margolin 2011). Moreover, this interleukin can suppress differentiation and maturation of dendritic cells both *in vivo* and *in vitro*, which can severely impair the immune response of the organism (Pinzon-Charry et al. 2005).

The present study showed a statistically significant increase of IL-6 secretion in MoDCs cultures generated from blood, spleens and lymph nodes of BLV-infected animals, compared to BLV-free controls. Similar results were obtained by Kabeya et al. (2001), Konnai et al. (2003) and Pyeon and Splitter (1998) in PBMCs of BLV infected animals. In addition, it has been shown that B cells freshly isolated from cattle with persistent lymphocytosis (PL) express very little IL-6 and IL-10, while after cultivation *in vitro* production of both those cytokines was significantly increased (Amills et al. 2004, Gillet et al. 2007). Additionally, it has been shown that serum of PL animals contains higher concentrations of IL-6 compared to both AL and BLV-negative animals (Trainin et al. 1996). These results were confirmed by Meiom et al. (1997), who demonstrated a significant increase in IL-6 level in the serum of animals infected with BLV. Szczotka et al. (2005) showed that serum from sheep experimentally infected with BLV exhibited higher concentrations of this interleukin than BLV-free controls. In the same study a correlation between IL-6 level and tumour development in sheep was observed (Szczotka et al. 2005). It has been proposed that the higher concentration of IL-6 in the blood of BLV infected cattle may be the result of lower affinity to the IL-6-receptor (Droogmans et al. 1994, Frie and Coussens 2015). A correlation between expression of this receptor and leukemogenesis in BLV infected cattle was not determined, but probably the elevated level of IL-6 is a consequence of this occurrence (Trainin et al. 1996). Multiple *in vitro* studies significantly added supplementary information about the role of IL-6 during BLV pathogenesis. It has been shown that cultures of PMBCs from leukemic animals exhibited a higher secretion of IL-6 in response to antigen stimulation. After addition of exogenous IL-6 to referenced cultures, expression of viral genes was dramatically reduced, which may suggest a contribution of this interleukin to the mechanisms of BLV latency (Meiom et al. 1997, Gillet et al. 2007). Additionally, both IL-6 and IL-10 were shown to be crucial for activation, differentiation and proliferation of B cells; for this reason they have been considered as factors contributing to lymphocytosis and transformation.

In the case of the same group originating from bone marrow, no significant differences were observed. This outcome may result from the natural properties of the tissue from which the DCs were generated. Bone marrow cells are at an early stage of development and are highly undifferentiated; DCs originating from this tissue may therefore lack some properties of fully mature DCs.

In contrast to MoDCs from naturally infected animals, the whole *in vitro* infected MoDCs group showed a significant decrease in IL-6 production similar to the case of IL-12. This occurrence may suggest a suppressed expression of the surveyed interleukins after *in vitro* infection.

IL-10 is anti-inflammatory cytokine, which takes part in the regulation and dissolution of immunological response (Pestka et al. 2004). IL-10 in humans and cattle mostly appear to have a down-regulating effect on both Th1 and Th2 clones. This occurrence is usually preceded by a reduction in the expression of proinflammatory cytokines by the whole T-cell population (Stephens et al. 2003). It has been shown that IL-10 can induce DCs apoptosis *in vitro* and substantially reduces the ability to effectively present antigens by suppression of DCs differentiation and maturation (Pinzon-Charry et al. 2005). Suppression of antigen presentation in DCs is a keystone of immunotolerance; however this property is often utilized by viruses to limit the immune response during infection (Wilson and Brooks 2011).

In the present study, in MoDCs obtained from blood, spleens and lymph nodes of naturally infected animals, a statistically significant increase in IL-10 secretion was observed. Also, in analogous bone marrow MoDCs cultures, elevation in the average level of this interleukin was noted, however in this case the difference was not statistically significant. Similarly to naturally infected DCs from the present study, increased IL-10 production has often been described in BLV-associated literature in reference to different cell populations. Pyeon et al. (1996) showed that PBMCs of leukemic cattle expressed a substantially larger amount of IL-10 compared to cells of BLV-free or aleukemic animals. Similar results were also observed in macrophages of PL cattle (Pyeon et al. 1996). Additionally, Jakobson et al. (1998, 2000) confirmed that, in contrast to other cytokines, an elevated level of IL-10 is a distinctive feature of the late stage of BLV infection; furthermore, a systematic increase in its concentration can indicate disease progression. Elevated concentration of IL-10 and its dependence on infection stage was also confirmed in the serum of experimentally infected sheep (Szczotka 2007).

In MoDCs generated from the spleen and lymph nodes of naturally infected cattle increased secretion of IL-10 and IL-6 (almost twice the value of controls in both cases) accompanied by a significant decrease in IL-12 level (both p40 and p70) have been observed. These results seem to be consistent with studies carried out on late stage leukemia. It has been shown that production of Th2 cytokines (mostly IL-10) in PBMCs of PL animals was increased, while



expression of Th1 (IL-2, IL-12, INF- $\gamma$ ) was reduced (Pyeon and Splitter, 1999). This switch in cytokine profiles from Th1 to Th2 has often been described as a characteristic feature of late stage BLV infection (Kabeya et al. 2001, Konnai et al. 2003, Usui et al. 2007). This occurrence can be very advantageous for the progression of BLV pathogenesis, and is not common for other *Retroviridae* such as HTLV and HIV (Clerici and Shearer 1994, Pyeon and Splitter 1999, Gillet et al. 2007). Also, analysis of HIV-infected DCs demonstrated a correlation between an increase in IL-10 production and disturbances in immunological mechanisms. As a result, impairment of DCs maturation with dominance of immature phenotype, responsible for immunological tolerance, over mature phenotype engaged in antigen presentation and control of immunological reaction, was observed. It has also been shown that intensified IL-10 expression suppresses production of proinflammatory cytokines IL-12 and IFN- $\gamma$ , which are crucial for induction of the Th1 response (Wilson and Brooks 2011).

On the other hand it has been shown that IL-10 may contribute to suppression of BLV spread in the organism. This interleukin can actively block BLV replication in adherent cultures of monocytes/macrophages and the whole process is dependent on macrophage factors (Pyeon et al. 2000). Additionally, it has been demonstrated that in PBMCs culture from cattle with persistent lymphocytosis was more prone to *in vitro* stimulation. PBMCs of BLV-positive cattle cultured with IL-10 showed decreased expression of both Tax and Pol BLV proteins, while in the case of IL-12 no impact on BLV expression was observed (Pyeon and Splitter 1999, Frie and Coussens 2015). Increased production during disease progression in connection with the suppression capabilities of IL-10 on viral protein expression may suggest its important role in BLV latency. The presence of a similar mechanism was also proposed in the case of HTLV (Gillet et al. 2007).

The results obtained from *in vitro* infected cultures indicated suppression of IL-10 expression in all analyzed DCs populations with the exception of blood-derived MoDCs. In this population tendencies to higher production of IL-10 were shown; however, the survey did not unequivocally confirm if this outcome resulted from individual differences or was related to BLV pathogenesis.

Elevated secretion of IL-10 and IL-6 by BLV-infected cells may suppress the host immune response to viral infection, especially in association with IL-12 depletion (Pyeon and Splitter 1999). The cytokine profile that indicates a shift in the balance of Th1/Th2 response may play a role in the control of

the viral infection and lead to viral persistence (Gillet et al. 2007, Frie and Coussens 2015). Multiple studies have confirmed that Th2 cytokines induce inhibition of cellular response and render cytotoxic lymphocytes and NK cells unable to eliminate the virus from the organism (Lucey et al. 1996). This is also consistent with the fact that animals with persistent lymphocytosis show a weaker cellular response to accompanying infections (Lundberg and Splitter 2000). Many reports suggest that impairment of DCs function after infection is crucial for HTLV pathogenesis and the present study seems to confirm this occurrence in the context of BLV infection.

## Conclusions

In the present study dendritic cells generated from the blood of BLV-positive animals were characterized by higher secretion of IL-6, IL-10 and IL-12(p40), compared to BLV-negative controls. In cultures of bone marrow MoDCs, no statistically significant differences were observed; this may be a result of the natural properties of the original tissue. In MoDCs originating from the spleen and lymph nodes of infected animals an increase in Th2 cytokine (IL-10 and IL-6) secretion and decrease in Th1 (both forms of IL-12) production was noted. This may suggest impairment of the immune system, rather typical for persistent viral infections. In cultures of MoDCs infected *in vitro* with BLV a significantly lower secretion of almost all the analyzed cytokines was observed.

On the basis of the results it can be concluded that BLV caused significant changes in cytokine secretion of dendritic cells. The changes in cytokine profile of different BLV-infected dendritic cell populations probably result from distinctive functions of these populations during the viral infection. Evidence indicates that BLV infection alters secretion and production of cytokines in response to stimuli. The current study definitively confirmed that BLV alters the cytokine profile of DCs and the observed imbalance may contribute to disease progression.

Additionally, the examined DCs populations, through higher production of IL-6 and IL-10, may play a contributory role in viral latency and oncogenesis.

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