Original article

Determination of cytokine profiles in populations of dendritic cells from cattle infected with bovine leukaemia virus

E. Iwan[1], M. Szczotka[1], J. Kocki[2], A. Pluta[1]

[1] National Veterinary Research Institute, Department of Biochemistry,
24-100 Puławy, Al. Partyzantów 57, Poland
[2] Medical University, Department of Clinical Genetics,
20-080 Lublin, Radziwiłłowska 11, Poland

Abstract

Dendritic cells (DCs) due to their ability to present antigens are essential during the immune response to infections. The aim of the study was to evaluate the influence of bovine leukaemia virus (BLV) infection on DC properties. Cytokine profiles of myeloid, plasmacytoid and monocyte derived DCs from BLV infected cattle were analysed. Concentrations of IL-6, IL-10, IL-12, IFN-γ, and TNF-α in DC cultures were measured by flow cytometry. Obtained results indicated activation of pDCs population, where a significant increase in production of the IFN-γ was shown. Meanwhile, a decrease in production of IFN-γ and increase in production of IL-10 were shown in mDCs; the main population responsible for antigens presentation. This may indicate a contributory role of the population during the process of persistent infection. In MoDCs population a significant elevation in secretion of proinflammatory cytokines – IL-6 and TNF-α was noted.

Key words: bovine leukaemia virus, dendritic cells, cytokines

Introduction

Bovine leukaemia virus (BLV) is closely related to the human T-cell leukaemia virus (HTLV-1). It is an aetiological agent of enzootic bovine leukosis (EBL), a lymphoproliferative disease with worldwide range. It has been shown that this deltaretrovirus persists in approximately 1% of peripheral blood cells, inducing asymptomatic infection (aleukaemic stage – AL) in most of BLV-positive cattle. Enzootic bovine leukosis, prevalently manifested by an accumulation of infected B-lymphocytes, called persistent lymphocytosis (PL) – affects about one-third of infected animals (Barez et al. 2015). In about 5%–10% of cases, BLV infection can evoke an oncogenic process, resulting in fatal leukaemia. Its most spectacular consequence is formation of mono- and oligoclonal tumours in internal organs (Lairmore 2014). B cells are the main target for the virus, but it also is able to infect other types of cells: T cells, monocytes, macrophages, and dendritic cells (DCs) (Iwan et al. 2014).

Many immunological processes are regulated on the level of DCs, which are especially important factors during response to pathogen infections. DCs have the capacity to take up, process, and present antigen to naive T-cells; that is why they are considered to be the most potent antigen presenting cells (APCs) in the immune system. They influence the type of T cell re-

Correspondence to: E. Iwan, e-mail: Ewelina.Iwan@piwet.pulawy.pl

sponse, based on the cytokine microenvironment in which the antigen is encountered. DCs also initiate and modulate the primary immune response, take part in the innate immunity against pathogens and induce the immunological tolerance (Banchereau and Steinman 1998). The surface of DCs contains large amounts of molecules essential for antigen presentation, *e.g.*: both MHC I and II complexes, CD40, CD80, CD83 and CD86 (Palucka and Banchereau 2012). There are two separate lineages of DCs: myeloid (mDCs) and plasmacytoid (pDCs) (Gibson et al. 2012). Depending on the lineage, origin and maturation stage, DCs have specific phenotype and function in the immune system. While mDCs, described also as conventional DCs, account mostly for the presentation of antigens, pDCs are responsible for induction of the immune tolerance, actively participate in anti-tumour immunity and create the first line of an antiviral response (Sei et al. 2014). CD14+ monocytes are fraction of precursors able to differentiate in to DCs (monocytes derived dendritic cells - MoDCs), creating convenient source of cells for *in vitro* studies and research. Considering their key role in the immune response, DCs are attractive target for viral infection. It was established that BLV may affect cells of the immune system on many levels; it can influence cell proliferation, apoptosis and expression of receptors and cytokines, which are especially crucial for the immune defence (Aida et al. 2013, Frie and Coussens 2015). The large part of DCs cell-cell signalling is mediated by cytokines and small proteins released in response to stimuli. Cytokines regulate growth, differentiation and sensitivity of cells, by which they can affect strength and length of the adaptive and innate immune response on multiple levels (Striz et al. 2014). Aberrations in cytokine network often play an important role in pathogenesis of persistent infections but also in oncogenesis of many tumours. It has been shown that BLV infection can cause changes in cytokine profile of immune system cells, but their impact on DCs is still largely unknown (Lairmore 2014, Frie and Coussens 2015).

Our previous preliminary investigations showed a significant influence of BLV on biology of DCs from lymphatic tissues (Iwan et al. 2017). Since DCs are functionally diversified group of cells, where distinctive populations in various maturation stages perform different roles during the immune response, additional broader approach to the subject was needed. In this study an impact of BLV infection on secretion profiles of three distinctive DCs populations from blood was examined.

The aim of the study was to investigate cytokine profiles in different DCs populations (mDCs, pDCs, and MoDCs) of cattle infected with BLV.

Materials and Methods

Animals and sample preparation - Samples of peripheral blood were collected during slaughter from 35 BLV-positive and 15 BLV-negative Polish Black-and-White Lowland breed cows. No experiments on live animals were conducted and no permission of Ethics Committee was required. The blood was collected from the jugular vein with EDTA/K_2 as anticoagulant. BLV infection was confirmed by ELISA (gp51 and p24 markers, IDEXX) and nested PCR (*env* gen). Additionally, haematological examinations of leukocyte levels and percentage compositions of white blood cells (Schilling formula) were performed, establishing all BLV-positive cattle as aleukaemic. BLV-negative animals served as a control group. Blood was centrifuged for 1 h at 8°C, 1,125 x g, pellet of leukocytes was collected, suspended 1:1 in PBS buffer (pH 7.2, Sigma-Aldrich), and centrifuged in histopaque gradient (Histopaque 1077, Sigma-Aldrich). Fractions of mononuclear cells were collected and counted. Three populations of DCs were obtained by bead-based immunomagnetic separation from blood mononuclear cells of each animal.

Isolation and generation of MoDCs - CD14+ monocytes were isolated from 1×10^7 mononuclear cells by immunomagnetic separation. The cells were suspended in isolation buffer (PBS, pH 7.2, 2 mM EDTA; Miltenyi Biotec, and 0.5% calf serum; Sigma-Aldrich) and incubated with CD14 MicroBeads (Miltenyi Biotec). Then magnetic separation was performed on LS Columns (Miltenyi Biotec), according to the manufacturer's instruction. CD14+ monocytes were collected and cultured in RPMI 1640 (Gibco) medium containing: 20% calf serum (Sigma-Aldrich), 0.3 mg/mL of L-glutamine (Sigma-Aldrich) and antibiotic-antimycotic solution (Sigma-Aldrich) diluted 1:100. Additionally, the cells were treated with 1:500 dilution of Granulocyte Macrophage Colony-Stimulating Factor (GM-CSF; Bio-Rad/Serotec) and 1 ng/mL of Interleukin 4 (IL-4; Endogen), then MoDCs cultures were generated after five days. DCs were cultivated in 10 mL culture flasks (Nunc) at 37°C, in 5% CO_2 and concentration of 0.2×10^6 cell/mL for one week.

Isolation of mDCs - Population of mDCs (CD11c+) was isolated from 1×10^8 peripheral blood mononuclear cells by immunomagnetic separation. The cells were suspended in 200 μL of isolation buffer, then 100 μL of blocking reagent (Miltenyi Biotec), 100 μL of CD19+ MicroBeads (Miltenyi Biotec) and 100 μL of CD11c+ biotin conjugated antibody were added. Fraction of CD19+ cells was removed during immunomagnetic separation on LS columns and mDCs were then coated with anti-biotin MicroBeads (Miltenyi Biotec) and separated. Isolated mDCs at concentration of 0.2×10^6

Table 1. Average cytokine concentrations in MoDC, mDC and pDC cultures.

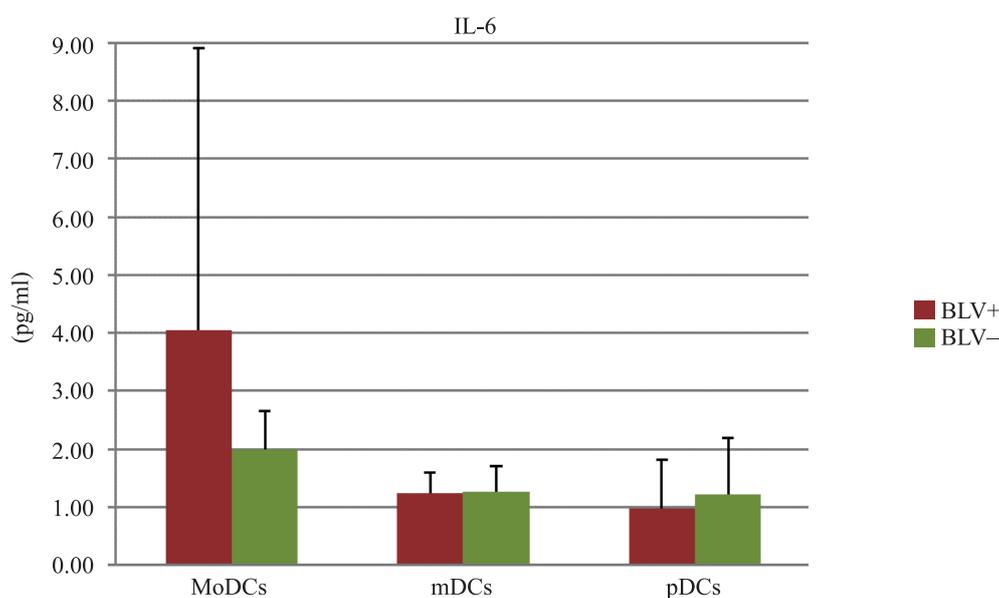| DC populations		IL-6	IL-10	IL-12 (p40)	IL-12 (p70)	TNF-α	IFN-γ
mDCs	BLV+	1.23	2.98	20.78	0.94	1.4	1.16
	BLV-	1.27	1.48	16.61	0.67	1.49	2.35
pDCs	BLV+	0.98	1.88	24.94	1.02	1.23	3.55
	BLV-	1.22	1.87	17.22	1.19	1.46	2.09
MoDCs	BLV+	4.05	3.65	49.6	0.73	2.6	2.3
	BLV-	2.0	0.68	22.28	0.65	1.39	1.6

Fig. 1. Average concentration of IL-6 in DC cultures.

were cultured in RPMI 1640 medium with 20% calf serum, 0.3 mg/mL of L-glutamine and antibiotic-antimycotic solution diluted 1:100. MDCs cultures were sustained in an atmosphere of 5% CO_2 at 37°C in 10 mL culture flasks (Nunc) for one week.

Isolation of pDCs - Population of pDCs (CD303+) was isolated from 1×10^8 peripheral blood mononuclear cells by immunomagnetic separation. The cells were suspended in 100 μL of isolation buffer, then 50 μL of blocking reagent and 100 μL of biotin conjugated CD303+ antibody (Miltenyi Biotec) was added. They were incubated at 8°C for 15 min. Then 150 μL of blocking reagent and 200 μL of anti-biotin MicroBeads were added to the mixture. Immunomagnetic separation and cultivation of pDCs was performed under the same conditions as described for mDCs.

Measurement of cytokine concentrations by flow cytometry - After a cultivation period, 5 mL of medium was collected from each of mDCs, pDCs and MoDCs cultures (0.2 x 10⁶cells/mL) and centrifuged for 10 min at 1,100 x g. Cell-free supernatants were then lyo-

philized and stored at –20°C until use. Directly before analysis, lyophilizates were resuspended in 1 mL of double-distilled water. Six populations of microbeads (BD Biosciences) with distinctive fluorescence and coated with monoclonal antibodies against: interleukin 6 (IL-6), interleukin 10 (IL-10), interleukin 12 p40 and p70 (IL-12 p40 and IL-12 p70), interferon γ (IFN-γ) and tumor necrosis factor α (TNF-α) were used during cytometric analysis. Standards of IL-6, IL-10, IL-12(p40), IL-12(70), IFN-γ and TNF-α (BD Biosciences) were prepared according to the manufacturer instruction. A total of 50 μL of each sample was added to 50 μL of microbeads mix (diluted 1:50; BD Bioscience) and incubated for 1 h at room temperature. Then 50 μL of detection mix (antibodies conjugated with phycoerythrin - PE, diluted 1: 50, BD Bioscience) was added and 2 h incubation in dark was performed. Microbeads were washed with 1 mL of PBS buffer (pH 7.0; Sigma-Aldrich), centrifuged 300 x g for 10 min and suspended in 300 μL of PBS buffer. The samples were analysed in flow cytometer Navios (Beckman Coulter) at 576 nm
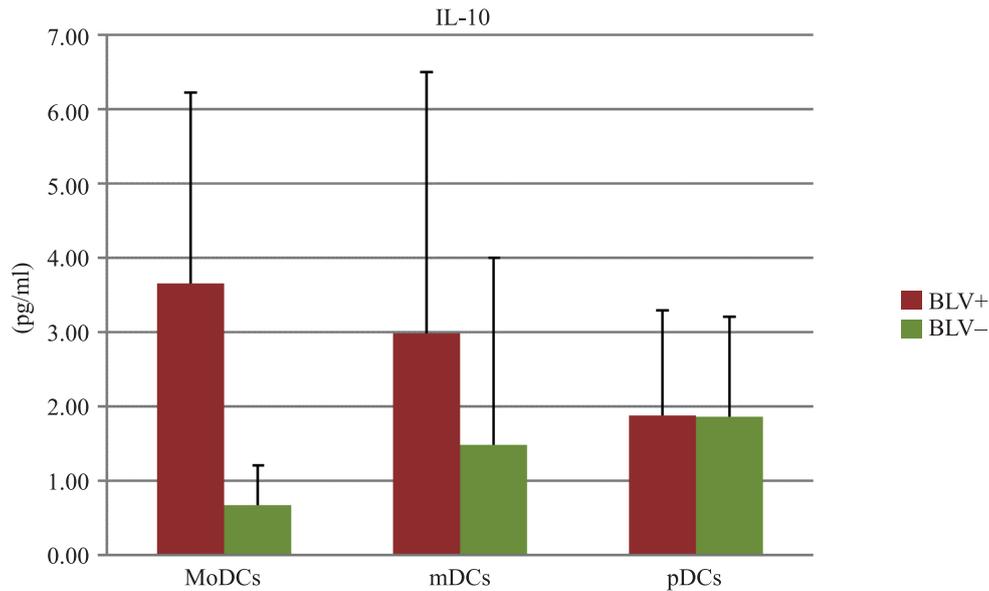
IL-10

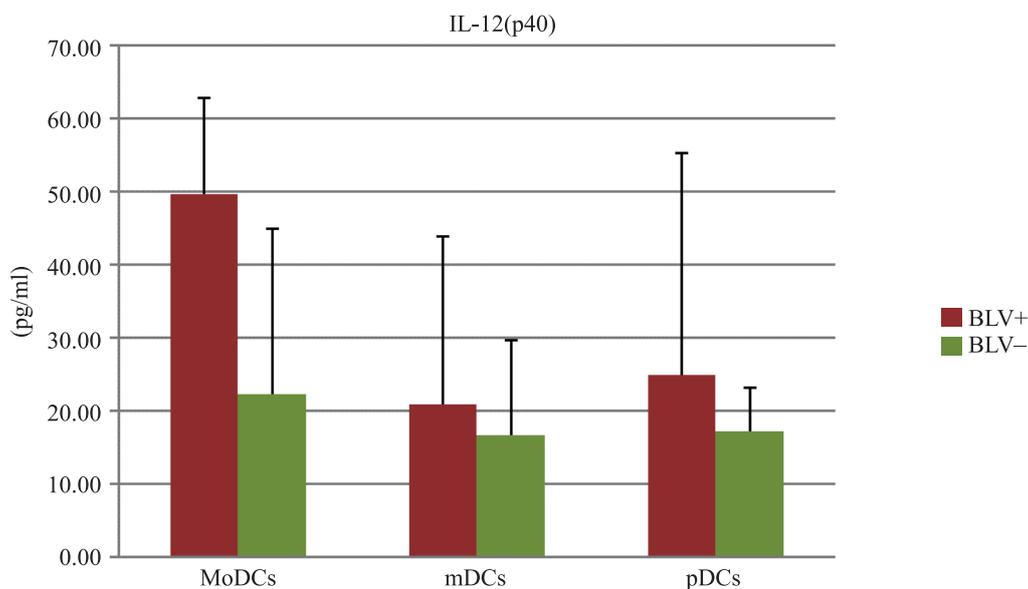Fig. 2 Average concentration of IL-10 in DC cultures.

IL-12(p40)

Fig. 3. Average concentration of IL-12(p40) in DC cultures.

(PE), 660 nm (Allophycocyanin – APC), and 680 nm (APC/Cyanine 7 - Cy7). Cytokine concentrations were calculated according to standard curves. Results were given in pg/mL calculated for 1×10^6 cells. Cultures of DCs from uninfected animals were used as a control. The statistical analysis was performed by U Mann--Whitney test with the use of Statistica 10 software, accepted p value was 0.05.

Results

A statistically significant increase in IL-10 production was observed in mDCs population originated from blood of BLV-infected cattle. The average concentration of IL-10 in this cell cultures amounted to 2.98 pg/mL, while in BLV-free controls it was 1.48 pg/mL (Fig. 2 and Table 1). In this group, a statistically significant raise in the IL-12(p70) level (0.94 pg/mL), compared to the control (0.67 pg/mL), was noted (Fig. 4 and Table 1). The mean concentration of IL-12(p40) in BLV-positive mDCs cultures was 20.78 pg/mL, while in analogous uninfected cultures it was 16.61 pg/mL (Fig. 3 and Table 1). The data showed higher concentration of IL-12(p40) in this group, but the results were not statistically significant. At the same time in BLV-infected mDCs decrease in IFN-γ levels was observed, those results were statistically significant.
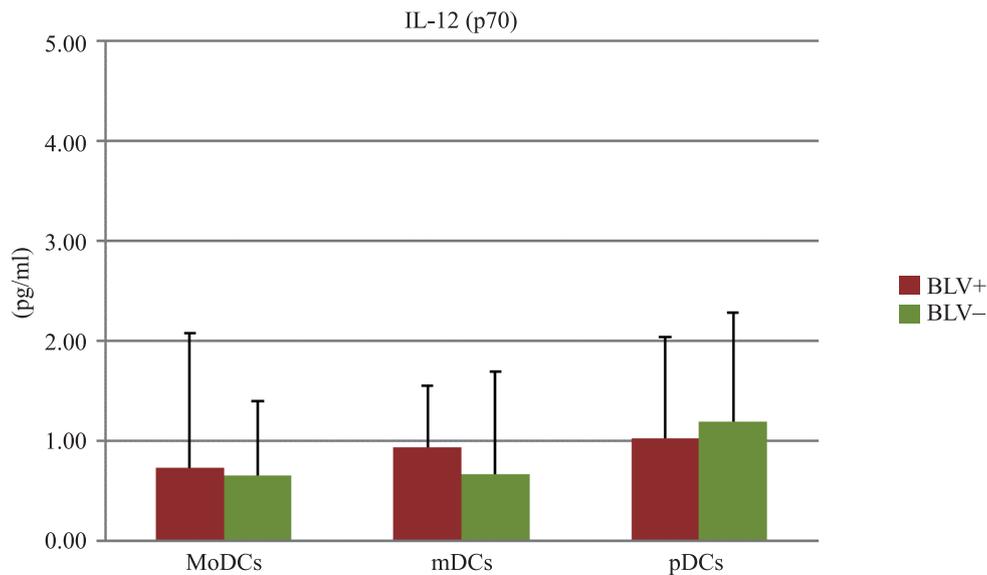
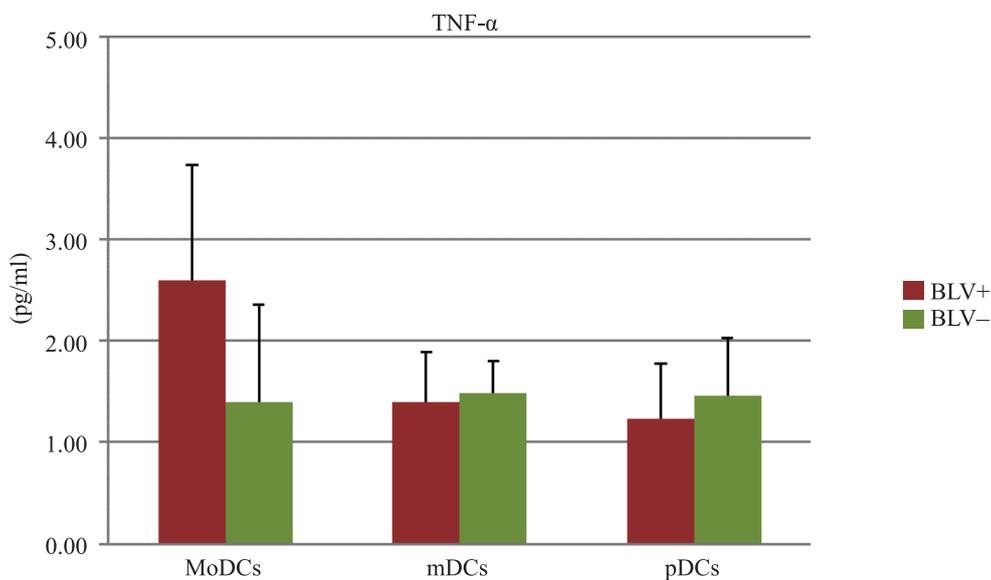Fig. 4. Average concentration of IL-12(p70) in DC cultures.

Fig. 5. Average concentration of TNF-α in DC cultures.

Its average concentration was 1.16 pg/mL in infected animals and 2.35 pg/mL in the control group (Fig. 6 and Table 1). In the case of IL-6 and TNF-α, differences were minimal and not statistically significant. In infected group, mean concentration of these cytokines was 1.23 pg/mL for IL-6 and 1.40 pg/mL for TNF-α, while in control group it was 1.27 pg/mL and 1.49 pg/mL, respectively (Figs. 1 and 5, and Table 1).

In population of BLV-infected pDCs statistically significant increase in IFN-γ secretion was observed. In these cultures the mean level of IFN-γ amounted to 3.55 pg/mL, while in corresponding BLV-free cultures it was 2.09 pg/mL (Fig. 6 and Table 1). Additionally, in this population rise of average level of IL-12(p40) was

24.94 pg/mL, as compared to controls – 17.22 pg/mL (Fig. 3 and Table 1). However, the difference was not statistically significant. The average concentration of TNF-α in BLV-infected pDCs cultures amounted to 1.23 pg/mL and this value was lower than in BLV-free cultures - 1.46 pg/mL (Fig. 5 and Table 1). This discrepancy tended to be statistically significant, when P-value was 0.1. The mean level of IL-6 in investigated group was 0.98 pg/mL, while in BLV-free culture 1.22 pg/mL (Fig. 1 and Table 1). Though the difference was not statistically significant, the results may suggest a decrease in secretion of IL-6 in BLV-infected population. In cultures from naturally infected cattle average concentrations of IL-10 and IL-12(p70) were
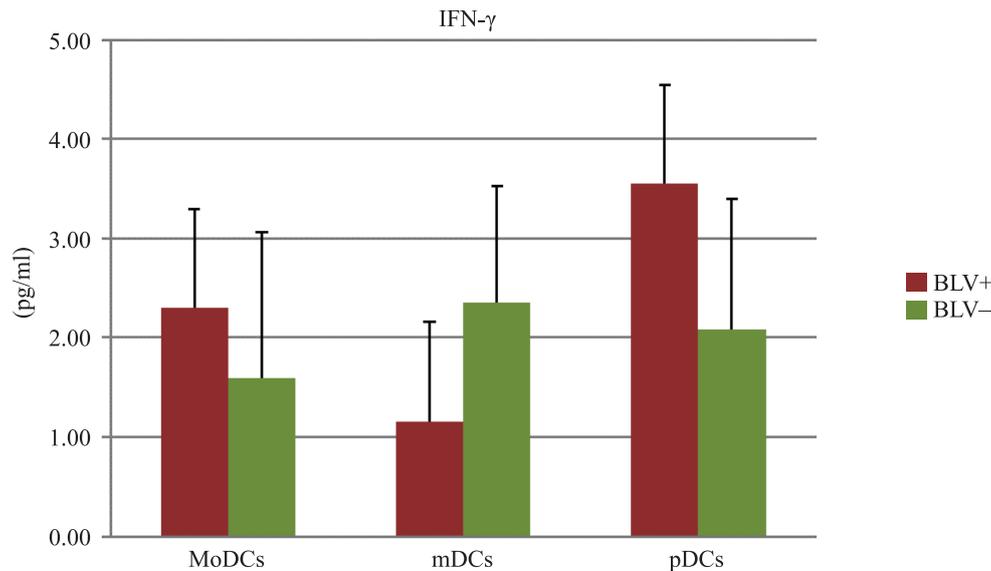
Fig. 6. Average concentration of IFN-γ in DC cultures.

1.88 pg/mL and 1.02 pg/mL, respectively. While in controls the concentrations were 1.87 pg/mL for IL-10 and 1.19 pg/mL for IL-12(p70) (Figs 2 and 4, and Table 1). In both cases no statistically significant differences were noted.

A statistically significant increase in IL-6 and TNF-α secretion was found in cultures of MoDCs from BLV-positive cattle. In this group the level of IL-6 averaged 4.05 pg/ml, while in uninfected cultures it was 2.0 pg/mL (Fig. 1 and Table 1). The mean concentration of TNF-α in BLV-positive cultures was 2.6 pg/mL, while in controls it was 1.39 pg/mL (Fig. 5 and Table 1). These results demonstrated almost double increase in TNF-α production in BLV infected group. In addition, average level of IL-12(p40) showed substantial increase (49.60 pg/mL), compared to BLV-negative controls (22.28 pg/mL) (Fig. 3 and Table 1). The observed difference tended to be significant at P = 0.1. The mean level of IFN-γ in infected cultures was 2.30 pg/mL, while in control group it was 1.60 pg/mL (Fig. 6 and Table 1). In this case results also tended to be statistically significant, when P-value was elevated to 0.1. Additionally, obtained results exhibited slight but not statistically significant increase in the average concentration of IL-12(p70) in BLV infected cultures (0.73 pg/mL) in comparison to controls (0.65 pg/mL) (Fig. 4 and Table 1). Also in BLV-positive MoDCs group, a substantial rise in IL-10 production was noted, but the difference was not statistically significant. The mean concentration of IL-10 in infected group amounted to 3.65 pg/mL, while in BLV-negative group it was 0.68 pg/mL (Fig. 2 and Table 1).

Discussion

BLV causes a persistent infection, in which the virus is not cleared but remains in cells, without rapidly killing or even producing excessive damage to the host. During the persistent infection expressions of both viral and cellular genes are modulated and, in consequence entire host immune response is alternated, often resulting in aberrations of cytokine network and immunosuppression. DCs are a key element of organism antiviral response, which make them an attractive target for viral infection. Moreover, if other *Retroviridae* (*e.g.* HIV or HTLV) are indicators, they can be not only infected but also actively participate in virus propagation (Pique and Jones 2012). Cytokine profile of DCs depends mostly on their origin, signals from environment and outside factors, *e.g.* pathogen infections (Stephens et al. 2003). Detailed investigation of secretion profiles can provide valuable data considering that DCs are still relatively unknown factor in BLV pathogenesis. In this survey production of six cytokines: IL-6, IL-10, IL-12(p40), IL-12(p70), IFN-γ, and TNF-α was investigated in DCs cultures from naturally infected cattle. This *in vitro* study showed distinct differences between secretion profiles of the analysed populations, which could significantly affect immune response *in vivo* and its outcome. Taking under consideration type of immune response, cytokines can be classified as Th1 (e.g. IL-2, IFN-γ, IL-12, TNF-β), and Th2 (*e.g.* IL-4, IL-5, IL-6, IL-10, and IL-13) (Lee and Margolin 2011).

IL-6 is generally regarded as a pro-inflammatory cytokine; however, it has also some anti-inflammatory and regenerative properties (Scheller et al. 2011). This cytokine is reported to be frequently deregulated

during neoplastic disease and is often used as a prognostic marker. The present study showed almost no deviation in the production of IL-6 between mDCs from naturally infected and BLV-free cattle. In contrast MoDC population showed a statistically significant increase in IL-6 production, while pDCs from BLV-positive animals tended to express less of this cytokine. An elevated production of IL-6 is quite often correlated with progression of BLV infection. For example, in peripheral blood mononuclear cells (PBMCs) of BLV-positive cattle increased expression of this interleukin was observed by Konnai et al. (2003). Additionally, sera of PL animals contain more IL-6 compared to AL- and BLV-negative cattle (Trainin et al. 1996). Furthermore, Szczotka et al. (2005) observed that sera of sheep experimentally infected with BLV had higher levels of interleukin 6. Also in this survey correlation between IL-6 level and tumour development was noted (Szczotka et al. 2005). In addition, it was shown that B cells freshly isolated from PL cattle express very little IL-6 and IL-10, but after *in vitro* cultivation, the production of both cytokines was strongly and rapidly up-regulated (Gillet et al. 2007). The results of *in vitro* studies in whole, suggest that this interleukin probably plays a contributory role in BLV latency (Gillet et al. 2007). The fact that IL-6 is able to suppress the differentiation and maturation of DCs is crucial for proliferation of B cell and may give evidence to its supporting effect on BLV progression and oncogenic transformation (Pinzon-Charry et al. 2005).

IL-10 was initially identified as a CD4 produced Th2 cytokine with the ability to indirectly repress Th1 responses (Moore et al. 2001). It has substantial regulatory properties, making this anti-inflammatory cytokine critical for dissolution of the immune response and prevention of tissue damage. In humans and cattle, IL-10 mostly appears to have a down-regulating effect on expression of proinflammatory cytokines (Stephens et al. 2003). IL-10 can abort T cell responses during priming, but also inhibits ongoing T cell activity to viral infections. Additionally, this cytokine can directly affect T cells by limiting their proliferation, functional differentiation and effector activity (Brooks et al. 2010). Presented study indicates an evident increase of IL-10 secretion in mDC and MoDC populations from BLV-positive cattle. In case of MoDCs the difference was not statistically significant, but it still may suggest the tendency to produce more of this cytokine. The results of our earlier studies seem to confirm this conclusion. Our preliminary survey indicated a significant elevation in IL-10 and IL-6 concentrations in MoDCs generated from blood, lymph nodes, and spleen of BLV-infected cattle (Iwan et al. 2017). Also the data reported by other authors indicate an increase

in the production of IL-10 during progression of BLV infection. It was noted that PBMCs of leukaemic cows express substantially larger amount of IL-10 compared to cells of BLV-free or aleukaemic animals. Similar results were observed in macrophages of PL cattle (Pyeon et al. 2000). It was confirmed that an increased expression of IL-10 is a distinctive feature of leukaemic stage of EBL and systematic raise of its level indicates disease progression. Moreover, elevated concentrations of IL-10 were noted in sera of experimentally infected sheep and this occurrence was associated with progression of disease in animals (Szczotka 2005). Furthermore, an increased secretion of IL-10 in mDCs from naturally infected cattle was accompanied by a decreased production of IFN-γ. A similar negative correlation between IL-10 and IFN-γ was observed by Tirziu et al. (2013) in sera of BLV infected cattle. This occurrence suggests a down-regulation of Th1 immune response and induction of immunosuppression, which often are typical for a late stage BLV infection (Konnai et al. 2003, Usui et al. 2007). This kind of disturbance in the equilibrium of a cytokine network can be very advantageous for the progression of BLV pathogenesis. IL-10 can actively block BLV replication in adherent cultures of monocytes/macrophages, suggesting contribution of this cytokine to mechanisms of viral latency (Pyeon et al. 2000, Gillet et al. 2007). Moreover, it was noted that IL-10 can actively suppress the differentiation and maturation of DCs. It inhibits the expression of stimulatory molecules (*i.e.*, MHC classes I and II, CD80, CD83, and CD86) and alters cytokine production. As a result process of antigen presentation is significantly constricted (Pinzon-Charry et al. 2005). Suppression of antigen presentation in DCs is a keystone of immunotolerance; however, these properties are often utilised by pathogens to limit immune response (Wilson and Brooks 2011). The existence of a similar mechanism was discovered in the case of many *Retroviridae,* such as HTLV and HIV (Gillet et al. 2007, Wilson and Brooks 2011). HIV-infected DCs show correlation between increased expression of IL-10 and disturbances in mechanisms of the immune response. As a result the impairment of DCs maturation occurs, causing an imbalance between immunological tolerance and antigen presentation. This event enhances viral propagation and is a main contributory factor in establishing of viral persistence.

The proinflammatory cytokine IL-12 is produced mostly by antigen-presenting cells, DCs in particularly. Interleukin-12 is essential for polarisation of näive T cells in Th1 cells and induction of IL-2 and IFN-γ secretion. IL-12 is a heterodimer (p70) combined of two subunits (p35 and p40). P40 has been shown to be secreted either as a homodimer or independently as

a monomer. Expression of IL-12(p70) and IL-12(p40) can differ, therefore, both of them are inspected separately. The current study showed increased secretion of both IL-12 forms in BLV-positive mDCs. While the difference in levels of p70 was small but statistically significant, in the case of p40 it was insignificant. In pDCs of BLV-infected animals the results indicated an increase in IL-12(p40) secretion. Additionally, no differences in the production of IL-12(p70) between infected and BLV-free populations were observed. MoDCs of BLV-positive cattle exhibited elevated production of both IL-12 forms. IL-12(p40) concentration was increased twofold in comparison to analogical BLV-free cultures. Increased secretion of IL-12 observed in DCs of naturally infected aleukaemic cattle seems to be consistent with the results obtained from PBMCs of AL animals by other authors. It was established that PBMCs of aleukaemic cattle express significantly more of this cytokine as compared to BLV-negative controls. However, often during progression of BLV infection, a significant reduction in IL-12 expression can be noted (Muller et al. 2003, Usui et al. 2007). Also our previous study concerning DCs showed a significant decline of IL-12(p40) and IL-12(p70) secretion in the lymph node and spleen DCs originated from BLV-positive animals, suggesting progression of infection in those cell populations. The down-regulation of IL-12 can be very advantageous for progress of BLV pathogenesis. It may indicate a dysfunction of APCs, leading to an impairment of the effector cells in innate and adaptive response (Konnai et al. 2003, Frie and Coussens 2015). Additionally, the results of present study indicated that in all analysed groups concentration of IL-12(p40) was higher than whole heterodimer p70. Analogous regularity was observed in our previous study concerning cytokine profiles of MoDCs from tissues of BLV infected cattle. This occurrence was also noted by Stephens et al. (2003), who suggested that elevated expression of IL-12(p40) in DCs cultures, rather than whole IL-12(p70) complex is an early host response to pathogen infection and that it may accounts for shifting T cell response in the direction of Th1 cytokine pattern. Additionally, it was noted that expressions of p40 and p35 subunits are regulated autonomously, where p35 subunit is more prone to inhibition by Th2 cytokines (especially IL-10) and is produced at much lower level than p40. While p40 is a biologically functional chemotactic cytokine with capability to activate DCs alone, it does not possess activity of p70 complex (Vignali and Kuchroo 2012). Moreover, p40 subunit can compete for IL-12 receptors, thereby indirectly impairing immune reaction (Méndez-Samperio 2010). Taking this fact under consideration, a significant increase in p40 production in BLV-infected MoDCs, while expression

of whole complex shows almost no difference, may indicate partial weakening of the immune response. Interferons are more important cytokines during early response to viral infections (Vignali and Kuchroo 2012, Lippitz 2013).

While DCs mostly secrete high quantities of I type interferons (IFN-α and β), some fractions of DCs from both myeloid and plasmacytoid lineages are able to produce small amounts of IFN-γ. As cytokine of Th1 response, IFN-γ takes part in polarisation of cytotoxic cells, activates macrophages and induces functional maturation of APCs (Stephens et al. 2003). IFN-γ creates positive feedback loop with IL-12 and exhibits similar antiviral and antineoplastic characteristic. This study showed a statistically significant decrease in IFN-γ secretion in mDCs of BLV-positive animals, while secretion of this cytokine in pDCs of infected animals was significantly elevated in comparison to controls. Also, increased production of IFN-γ was observed in BLV-infected MoDCs but in this case the difference was not statistically significant. Similar results, confirming elevated expression of interferon γ in PBMCs of BLV-infected animals were published by Usui et al. (2007). They determined that PBMCs of sheep experimentally infected with BLV and without symptoms of clinical leukaemia showed a significant increase in IL-12 and IFN-γ production, while production of IFN-γ in sheep with leukaemia was drastically decreased. The same authors described the existence of connection between IFN-γ expression-indicator of strong immune response and suppression of virus spread in an early stage of infection (Usui et al. 2007). Ohira et al. (2016) noted that cultures of T cell and NK from cattle infected with BLV show a significant decrease in levels of IFN-γ and TNF-α, correlating with increasing proviral load and progressing infection. Many studies on PBMCs of persistently infected cattle showed the evidence of correlation between higher viral load and decrease in production of IFN-γ (Okagawa et al. 2012, Ohira et al. 2016). The antiviral potential of IFN-γ was demonstrated during its application to BLV-infected animals, resulting in activation of γδ T cells. It was also shown that this cytokine can suppress BLV replication *in vivo* (Murakami et al. 2004). In the present study, a decrease in IFN-γ levels in mDCs was accompanied by an increase in IL-10 production. Also, it was shown that a decrease in concentration of IFN-γ in sera of BLV infected cattle was negatively correlated with a level of IL-10, suggesting Th1 suppression (Tirziu et al. 2013). Our previous study also indicated an increased expression of IL-10 in DCs cultures from the spleen and lymph nodes of BLV-infected cattle. However, in this case it was correlated with the reduction of IL-12 production (IFN-γ was not examined), but this

correlation in case of mDCs was not observed. Stephens et al. (2003) suggested that IL-10 in cattle firstly inhibits IFN-γ expression, then secondly IL-12 production. Peyon et al. (2000) showed that PBMCs freshly isolated from leukaemic cattle express less IFN-γ, while PBMCs of aleukaemic cattle produce more of this cytokine even in comparison to BLV-free animals. It was clearly exhibited that progression of BLV infection correlates with a disturbance in the cytokine networks. The elevated expression of IL-10 may significantly reduce production of Th1 cytokines (IFN-γ, IL-12), resulting in inhibition of the immune response (Wilson and Brooks 2011).

TNF-α is both pro-inflammatory and regulatory cytokine engaged in processes of the immune response to the viral and bacterial infections (Frie and Coussens 2015). The main function of TNF-α described as acute-response cytokine is the up-regulation of multiple pro-inflammatory: chemokines, cytokines, adhesion molecules, and growth factors (Lippitz 2013). This study showed significant differences in TNF-α secretion in pDC and MoDC populations. Therefore pDCs of BLV-positive animals indicated decrease in TNF-α production, while MoDCs showed substantial increase in TNF-α secretion. A significant increase in TNF-α concentration in sera of BLV-infected cattle, compared to controls, was also observed by Werling et al. (1998). Furthermore, Usui et al. 2007 showed link between TNF-α expression and proliferation of BLV-infected B cells. Ohira et al. (2016) indicated that *in vitro* cultures from cattle infected with BLV show a decrease in levels of TNF-α, correlated with progressing infection. TNF-α is an important growth factor during spontaneous proliferation of PBMCs of PL cattle (Konnai et al. 2006). It was shown that PBMCs culture of leukaemic cattle secreted high amounts of proinflammatory cytokines, TNF-α and IL-6, as a result of antigen (gp51 and LPS) and ConA stimulation. On the other hand, TNF-α is considered to be cytokine inducing death of B cells and proliferation of BLV-infected B cells. It is also implicated in the pathogenesis of chronic inflammatory disorders and, by stimulating tumour growth, in the progression of leukaemogenesis (Lippitz 2013). Chronic inflammation and persistent infection produce a tumour-supporting microenvironment which is indispensable during the oncogenic process. Many clinical and epidemiologic studies have suggested a strong association between chronic infection, inflammation, and cancer (Lippitz 2013). Important components in this linkage are cytokines produced by activated innate immune cells. It is crucial to determine the specific role of different subsets and maturation stages of DCs during BLV pathogenesis. Understanding of these mechanisms may allow us to identify new targets for the treatment and prevent infectious diseases. Some of these regulatory mechanisms might also be involved in the immunosuppressive environment seen in tumours; therefore this study could provide new insights into cancer immunology.

Conclusions

The study showed that BLV infection differently influences analysed DC populations. In BLV-positive pDCs, the secretion of IFN-γ was significantly higher, which may suggest participation of this population in initiation of Th1 response. But despite the activation of this cell group, it should be remember that this DCs fraction is particularly responsible for immunotolerance, a key factor during a persistent infection. In the case of conventional mDCs, which are responsible for antigen presentation, an increased production of regulatory cytokine, IL-10 and a decreased production of IFN-γ could clearly be noted. This occurrence suggests inhibiting influence of BLV infection on mDCs, leading to limitation of antigen presentation and a down-regulation of T cell differentiation. In consequence it often results in suppression of immune response, higher immunotolerance for infected cells, and overall progression of EBL pathogenesis. Furthermore, results obtained from BLV-infected MoDCs were characterised by a significant increase in production of IL-6 and TNF-α. While induction of these proinflammatory cytokines may indicate activation of this population during BLV infection, they also have often been linked with oncogenic process, suggesting contributory role in leukaemogenesis - especially in connection with enhance expression of IL-10.

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