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The Isolation of BDCA-1 Myeloid Dendritic Cells from Bovine Blood for Detection of the Bovine Leukaemia Virus

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Abstract

This article is focused on the isolation of CD1c+ dendritic cells (DCs) from bovine blood and their using for detection of bovine leukaemia virus. DCs are able to initiate an immune response because of that are antigen-presenting cells. DCs also play a key role in the regulation of innate immunity (NK cell function). Reciprocally, NK cells affect the activity of DCs. DCs have a few sub-groups. Myeloid DCs have two subsets – CD1c+ (BDCA-1) and CD141 (BDCA-3). We focused on detection of the glycoprotein gp51 in BDCA-1 cells infected by bovine leukaemia virus using immunofluorescence. We were able to detect bovine leukaemia virus in DCs which occurs within 8 years of age in cattle and it is the serious disease that needs to be detected in time.

1. Introduction

Dendritic cells (DCs) are heterogeneous cell type with long protrusions that are able to present antigen to T lymphocytes (Abbas *et al.*, 2017). These cells are formed in the bone marrow and get into the tissues through the blood. Immature portions of DCs have low expression of costimulatory molecules on their surface and are unable to present antigen to T lymphocytes. Only mature DCs can do this and have a high production of cytokines (Ganguly *et al.*, 2013). DCs represent a connection between innate and adaptive immunity. DCs are divided into several subsets. Each subset has a different area of immunity and functions (Kratochvilova and Slama, 2018).

Cells expressing CD1c are a subtype of DCs, BDCA-1. They are developed from myeloid precursors in the bone marrow and make up about 0.5% of blood leukocytes (Peiser *et al.*, 2003). The antigen CD1c is a part of transmembrane glycoproteins that have the similar structure as the major histocompatibility complex (MHC) proteins. CD1c+ DCs express CD14 in small amounts. These cells were firstly described as negative for markers as CD16, CD20, and for CD141 (BDCA-3) (Schroder *et al.*, 2016). It was also described the same for CD303 (BDCA-2) and CD304 (BDCA-4) in human (Schroder *et al.*, 2016). BDCA-1 cells are able to express CD14 and CD11b, myeloid markers (CD13 and CD33) and Fc receptors (CD32 and CD64) (Peiser *et al.*, 2003).

To achieve isolation of BDCA-1 DCs, it is necessary to remove BDCA-1 expressing B lymphocytes. This can be attained through

binding CD19 MicroBeads with these B cells. Afterwards, myeloid DCs can be separated using CD1c (BDCA-1)-Biotin and Anti-Biotin MicroBeads.

Bovine leukaemia virus (BLV) is a C-type Retroviridae family and can cause enzootic bovine leukosis (EBL). The proliferation of T and B lymphocytes is induced by BLV in cattle (Szczotka *et al.*, 2019). BLV has the same symptoms as human T-cell leukaemia virus (HTLV-1, HTLV-2) and T-cell leukaemia virus of simian (STLV). BLV can occur in cattle aged 1-8 years. During infection of BLV, the sensitivity of T lymphocytes was increased due to immunoaffinity depletion of B lymphocytes and monocytes from peripheral blood or during the examination of positive T lymphocytes selection using immunomagnetic beads (Szczotka *et al.*, 2019). The nature and degree of activation of DCs are influenced by the antigen and the microenvironment in which the activation takes place. Various microbial components and cytokines are involved in that process. The mode of activation of DCs and their interaction with T lymphocytes determines the type of subsequent specific immune response (Crucian *et al.*, 2016). Moreover, DCs can be infected by BLV.

In this study, we used identification of protein gp51 for detection of BLV infected BDCA-1 cells. Gp51 is envelope glycoprotein of BLV which was previously detected by ELISA, PCR of flow cytometry (Szczotka *et al.*, 2012). We set a goal to identify gp51 in DCs by immunofluorescence as comparative detection for previously described methods.

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2. Material and methods

2.1 Animals

The study was investigated on 6 cows naturally infected with BLV. Cows were Polish Black and White Lowland breeds and in the age 4-9 years. Uninfected cows were set up controls (6 cows).

2.2 Detection of BLV

Blood samples were obtained from cows (infected and healthy), collected from *vena jugularis externa*. The BLV was detected by ELISA using commercial kits and by PCR method previously described (**Kuckleberg et al., 2003**).

2.3 Isolation of peripheral blood mononuclear cells (PBMCs)

For this purpose, histopaque solution (density 1.077 g/ml) was used. Ethylenedi-aminetetraacetic acid (EDTA) was used as an anticoagulant. Blood was centrifuged as previously described by **Szczotka et al. (2009)** and **Kratochvilova et al. (2019)** in their studies. After that, the buffy coat with some red blood cells and plasma cells were collected together. Histopaque was added to the tube where the buffy coat was layered. Centrifugation of the test tube was 2000 rpm (1 hour) (**Szczotka et al. 2009; Kratochvilova et al., 2019**). After centrifugation, there were acquired a layer of PBMCs.

2.4 CD1c+ (BDCA-1) isolation from PBMCs

In this study, there were used MACS CD1c+ (BDCA-1) Dendritic Cell Isolation Kit (Miltenyi Biotec, Germany) for isolation of CD1c+ DCs. We prepared 200 µl MACS buffer [Phosphate-buffered saline (PBS) and 0.5% bovine serum albumin (BSA)] and 2 mM EDTA where cells were resuspended. CD19 MicroBeads and CD1c-Biotin and FcR Blocking Reagent was added to the cell of suspension. Cells were mixed, incubated and after that washed (MACS buffer). Next, the cells were centrifuged and resuspended again. MACS Separator and the magnetic field were used. LD Column was added to the magnetic field and wash by 2 ml MACS buffer (Miltenyi Biotec, Germany). Suspension of the cell was layered onto the column. Cells with CD19 MicroBeads have stayed and other cells were passed through and collected. We washed this column. Other cells were resuspended. We added Anti-Biotin MicroBeads and cell were mixed and incubated (about 8 °C and 15 minutes). An MS Column was added to the magnetic field of MACS Separator with MACS

buffer and the cell was layered onto the column. CD1c-Biotin and Anti-Biotin were used for the labelling of CD1c+ cells. Unlabeled cells were removed, and cells labeled by MicroBeads stayed in the column. We removed the column from the separator and added to 15 ml falcon tube. After that, MACS buffered was added and we obtained CD1c+ cells. We used the same method as **Schroder (2015)** described in own work.

2.5 CD1c+ culture

The isolated CD1c+ were cultured at a concentration of 1x10⁶ cells/mL in standard culture flasks in RPMI 1640 medium [containing 1 % L-glutamine, 1 % penicillin-streptomycin, 10 % of fetal calf serum (CSF)] at 37 °C and in a humidified 5 % CO₂ atmosphere.

2.6 BDCA-1 cytology and immunofluorescence

Cultured cells were gentle pipetting, washed and added onto slides and air-dried during at 23 °C (room temperature). Nucleus of cells were labelled by DAPI.

BDCA-1 cells were fixed by acetone after air drying for 10 minutes and 4 °C. Gp51 monoclonal mouse antibody diluted 1:100 was added. This process took 30 minutes at 4°C in the dark. After washings, the slides were covered with FITC-labelled goat anti-mouse monoclonal antibody diluted to 1:300 and incubated for 30 minutes at 4°C in the dark. After that, the slides were washed, buffered glycerol was dropped onto the slides. For immunofluorescence analysis, there were used a fluorescence microscope (Olympus Life Science, Tokyo, Japan).

3. Results

The aim of this experiment was to isolate CD1c+ (BDCA-1) DCs from the blood and visualize gp51 as the marker of BLV. For this experiment, we used blood from cattle. After magnetic separation of cells, an enriched fraction of marked cells was obtained. CD1c+ cells were labelled by fluorescence on the gp51. Cells expressing the BLV gp51 protein were labelled by green fluorescence (FITC) and their presence was confirmed by immunofluorescence. CD1c+ cells had high cytoplasm and irregular spherical nucleus (see in Figure 1). It is typical for the type of these cells.

As the results shown, we were able to see labelled gp51 in BDCA-1 cells from infected cows with BLV (Figure 1). In control group, there were found no gp51 in mentioned cells.

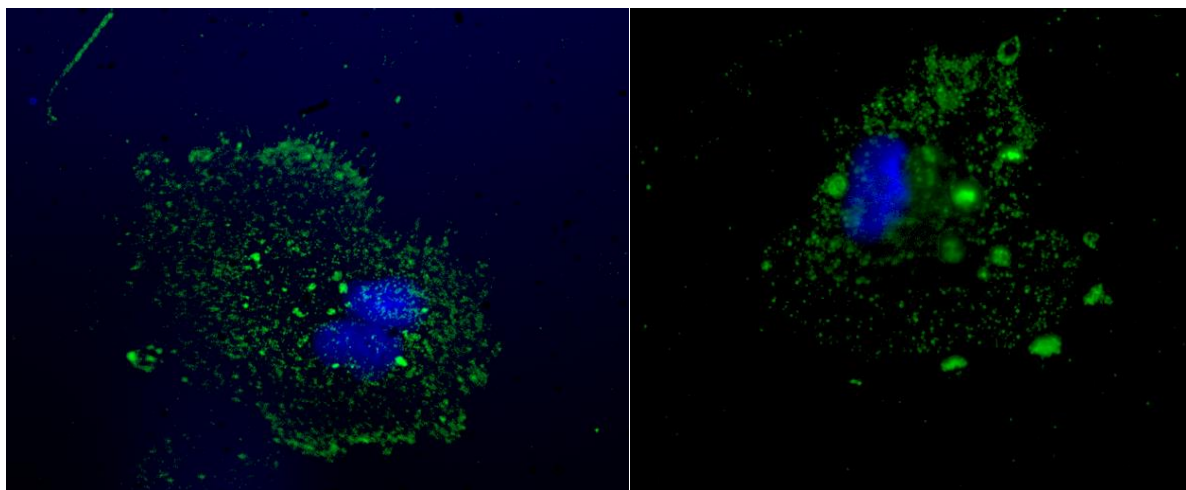


Figure 1 Presence of BLV gp51 in BDCA-1 cells. Green colour = gp51. Blue colour = DAPI (nucleus).

4. Discussion

The aim of this study was to evaluate if it is possible to use blood DCs for detection of BLV. We investigated this hypothesis on naturally infected cows with BLV. There were obtained blood from infected cow. Then, we isolated myeloid DCs – BDCA-1 and labelled those cells by antibodies against gp51 which is reliable marker of BLV. We are able to confirm that this method is suitable for detection of BLV in blood.

Blood DCs represent less than 1% of circulating PBMC and these cells are antigen-presenting cells with high expression of MHC (Hart *et al.*, 1997). Other authors refereed that this population is as large as 1-2% (Garner *et al.*, 2003). One cell subtype of blood myeloid DCs represents BDCA-1 which is CD1c positive cell population. It is possible to isolate this small population of DCs directly from the blood by a relatively simple technique (isolation of PBMCs and then isolation of Cd1c+ cells by magnetic separation using magnetic microbeads) (Szcotka *et al.*, 2009, Kratochvilova *et al.*, 2019).

Gp51 is surface envelope glycoprotein of BLV (Mamoun *et al.*, 1990; Marawan *et al.*, 2021). This glycoprotein has a crucial role in the viral life cycle. It contains the recognition site required for viral entry into the host cell (De Brogniez *et al.*, 2015; Marawan *et al.*, 2021). Therefore, gp51 is obvious marker of BLV in cells. Szcotka *et al.* (2019) showed in their work, that cows with BLV have increased expression glycoproteins of gp51, and p24 as structural protein of BLV.

BLV is also able to entry into DCs. Gp51 of BLV was detected in leukaemic DCs by flow cytometry (Szcotka *et al.*, 2012). Therefore, we wanted to try to analyze if it is also possible to detect gp51 in blood myeloid DCs (BDCA-1) by fluorescent microscopy. For that reason, the BDCA-1 cells were labelled by DAPI (to visualize the nucleus of cells) and by anti-gp51 antibody (FITC) to confirm the gp51 in infected BDCA-1. Our results showed that this method of labelling is suitable for detection of gp51 in infected BDCA-1.

That explained method can be additional method for detection of infected cells by BLV. This method properly add methods like PCR or flow cytometry. Using of more than one method is important for elimination of false positive or false negative results.

5. Conclusion

The virus BLV can be detected by the fluorescence in blood DCs. There is a lack of studies focusing on DCs in cattle as most research effort in this area is aimed at DCs in human. Data acquired by this experiment can, therefore, be of high value to

the scientific community and the explained method is good enough to complete methodology of BLV detection in cattle.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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