UTILISATION OF NON-SPECIFIC ANTIBODIES TO DETECT GOAT LEUKOCYTE POPULATIONS BY FLOW CYTOMETRY

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Introduction

Identification of different leukocyte populations is considered a very important step since it can serve as a first approach toward a correct diagnosis in veterinary hematology. The classical way to identify each leukocyte population is the microscopic one that involves a peripheral blood smear being analyzed. Automatic techniques are also available for this purpose showing a great accuracy in leukocyte differentiation. Usually goat leukocytes are identified as in other mammals due to their membrane and nucleus characteristics. Flow cytometry represents a relatively new technique used in veterinary medicine for different purposes including leukocyte differentiation with and without use of antibodies (1), identification and staging of neoplastic disorders mainly in dogs and cats (2), Ki67 index (3), DNA content (4), allergies and other medical conditions. In order to correctly identify each leukocyte population, use of species specific antibodies may be necessary. However if a different species specific antibody is used in another species, in some cases, a positive signal can be detected. To make this the antibody specificity and reactivity of one species should be proven to another species. The aim of this study was the evaluation of different species specific reactivity in goat leukocytes.

Material and Methods

Peripheral blood samples from healthy goats were collected in 2.5 ml Ethilendiamintetraacetat tubes. Samples were then sent to the Laboratory of Public Safety at the Faculty of Veterinary Medicine/Agricultural University of Tirana. All samples were processed using an Attune NxT flow cytometer within 24h from sampling. Briefly four non species specific antibodies were used: CD11b (Clone M1/70) conjugated PeCy5, CD5 (Clone: YKIX322.3) conjugated FITC, cocktail CD4/CD8 (YKIX302.9/ YCATE55.9) conjugated FITC/PE (tab 1). The adequate quantity of peripheral blood (106) was placed in flow cytometry tubes with the adequate quantity of the antibody. Incubation took place for thirty minutes at dark in 40C. A RBC lysis step was performed in order to exclude red blood cells from the analysis. Centrifugation of samples was done, the supernatant discarded and finally the remaining cells were resuspended in 150 µl of phosphate buffered saline. In order to compare the performances of antibodies the Stain Index was calculated in all cases using the following formula: MFI of Positive – MFI of Negative / 2* SD of Negative where MFI = median fluorescence intensity and SD = CV * Mean Negative / 100) (5,6).

Table 1 List of antibodies used to identify goat leukocytes

Antibody	Clone	Conjugation	Target Cells	Species Reactivity
CD5	YKIX 322.3	FITC	Mature T-Cells	Dog
CD4	YKIX 302.9	FITC	T-Helper Cells	Dog
CD8	YCATE 55.9	PE	T-Cytotoxic Cells	Dog
CD11b	M1/70	PECy5	Myeloid Cells	Mouse

Result and Discussion

In total ten cases were collected in this preliminary study. A positive signal was detected only for CD11b, while no positive signal was detected for CD5,CD4/CD8. In the last case no signal was detected meaning that no link between antibodies and antigens were present. Regarding CD11b it showed a positive signal in all cases for Granulocytes. In figure 1 results are showed in flow cytometry dot plots.

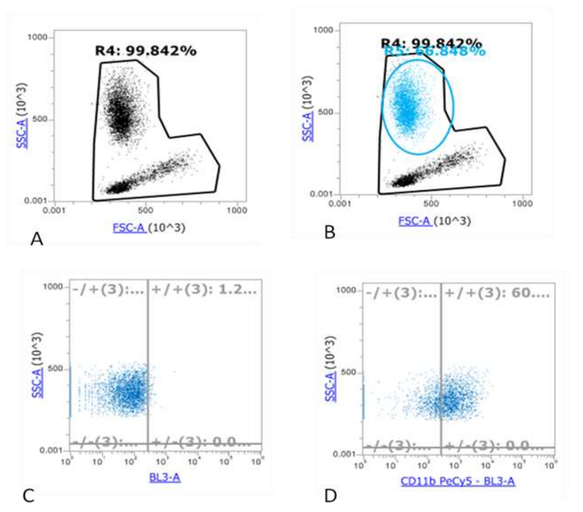

Figure 1. Dot plot showing reactivity to CD11b. A-Gate to include only leukocytes, excluding debris, B-Blue gate designed to select only Neutrophils, C-Neutrophils in third channel of fluorescence without antibody and D-Positive signal of Neutrophils against CD11b.

The antibody CD11b can be used to identify goat Neutrophils with a satisfactory accuracy. However low number of cases can be a lack of this preliminary study. Thus further investigation are warranted to confirm these results.

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