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THE IMMUNOPHENOTYPE OF CAMEL BLOOD EOSINOPHILS

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ABSTRACT

The aim of the present study was to use staining with monoclonal antibodies to different cell surface molecules and flow cytometry to analyse the expression pattern of cell markers on camel blood eosinophils. Based on their light scatter charecterestics and green autofluorescence, camel eosinophilic granulocytes were identified as $SSC^{high}/FSC^{low}/Fl\text{-}1^{high}$ cells within the granulocyte population. In comparision to neutrophilic granulocytes, camel eosinophils showed higher abundance of the cell surface molecules CD45, CD44, and CD11a but lower abundance of the cell markers CD172a and CD14. Collectively, the findings of the current study suggests a similar phenotype of camel, human, and bovine eosinophils.

Key words: Adhesion molecules, camel, eosinophilic granulocytes, flow cytometry, immunophenotype

The immunophenotype of eosinophils has been investigated for different species (Magyar *et al,* 1995; Pelan-Mattocks *et al,* 2001; Ramirez *et al,* 2018; Hassani *et al,* 2020; Oliveira *et al,* 2020). In humans, eosinophils differ from neutrophils by higher side light scatter (SSC), lower forward light scatter (FSC), negative CD10 and CD16, and dimmer CD11b, CD11c, CD13, CD15, and CD33 (Gorczyca *et al,* 2011). Flow cytometric analysis identified porcine eosinophils as positive for LFA-1 (CD11a/CD18) and swC3, a common marker of swine monocytes, granulocytes and macrophages, with no reactivity with antibodies recognising swine CD2, CD4, CD8 or MHC class II cell surface molecules (Magyar *et al,* 1995). In the dromedary camel, little is known about the phenotype and function of eosinophils. The number of camel blood eosinophils in blood ranges between 0.38 and 1.0 cell/µl blood, with higher numbers in adult animals than in newborn calves (Gaashan *et al,* 2020).

Flow cytometry has been widely used in humans and other species for the differentiation of leukocyte subpopulations on the basis of di fferences in cell size (as measured by forward light scatter), intracellular complexity (as measured by side light scatter), and intensity of fluorescence after staining with monoclonal antibodies to different cell markers (Appay *et al,* 2008; Gorczyca *et al,* 2011; Yu *et al,* 2016; Hussen *et al,* 2019). The aim of the present study was to use flow cytometry to analyse the expression pattern of cell markers on camel blood eosinophils.

Materials and Methods

Blood was obtained by venipuncture of the vena jugularis externa into vacutainer tubes containing EDTA (Becton Dickinson, Heidelberg, Germany) from 20 adult dromedary camels (*Camelus dromedarius*) aged between 8 and 12 years. All experimental procedures and management conditions used in this study were approved by the Ethics Committee at King Faisal University, Saudi Arabia (Permission number: KFU-REC/2020-09-25).

Isolation of leukocytes from camel blood

Separation of camel leukocytes was performed after hypotonic lysis of blood erythrocytes as described previously (Hussen *et al,* 2017). Briefly, blood was suspended in distilled water for 20 sec and double concentrated PBS was added to restore tonicity. This was repeated (usually twice) until complete erythrolysis. Separated cells were finally suspended in MIF buffer (PBS containing bovine serum albumin (5 g/L) and NaN_3 (0.1 g/L)) at 5 x 10^6 cells/ml. Cell purity of separated leukocytes was assessed by flow cytometry according to their FCS/SSC properties and always exceeded 90%. The mean viability of separated cells was evaluated by dye

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exclusion (propidium iodide; 2 µg/ml, Calbiochem, Germany) and it was above 90%.

Monoclonal antibodies

Monoclonal antibodies used in this study are listed in Table 1.

Table 1. List of antibodies.

Antigen	Antibody clone	Label	Source	Isotype
CD45	LT12A	-	mIgG2a	WSU
CD44	LT41A	-	WSU	mIgG2a
CD11a	G43-25B	-	mIgG2a	BD
CD172a	DH59b	-	mIgG1	WSU
CD14	TÜK4	-	WSU	mIgG1
CD163	LND68A	-	Kingfisher	mIgG1
mIgG2a	polyclonal	PE	Invitrogen	gIgG
mIgG1	polyclonal	FITC	Invitrogen	gIgG

Ig: Immunoglobulin; m: mouse; g: goat, FITC: Fluorescein isothiocyanate, PE: Phycoerythrin.

Membrane immunofluorescence and flow cytometry

Separated leukocytes (2 x 10^5) were incubated with different combinations of unlabeled primary monoclonal antibodies (mAbs) specific for the cell markers, CD45, CD44, CD172a, CD14, CD163, and CD11a in MIF buffer [membrane immunofluorescence buffer consisting of PBS containing bovine serum albumin (5 g/L) and NaN_3 (0.1 g/L)] (Hussen and Schuberth, 2017). After incubation (15 min; 4°C), the cells were washed twice and incubated with mouse secondary antibodies (IgG1, IgG2a; Invitrogen) labeled with FITC and PE, respectively. Washed cells were analysed using the Accurie C6 flow cytometer (BD Biosciences). At least 10^5 total leukocytes were collected and analysed with the CFlow Software, Version 1.0.264.21.

Statistical Analyses

Statistical analysis was carried out using the software Prism (GraphPad software version 5). Results are expressed as mean ± S.E. of the mean (SEM). Differences between means were tested with one-factorial analysis of variance (ANOVA). Results were considered statistically significant at a p-value of less than 0.05.

Results and Discussion

Eosinophilic granulocytes are innate myeloid cells with several important roles in both innate and adaptive immunity (Jacobsen *et al,* 2012; Furuta *et al,* 2014). Especially in the immune response to parasitic infections and in allergic reactions, eosinophils represent a characteristic cell type (Ramirez *et al,* 2018). In dromedary camels, the immunophenotype of blood eosinophils has not been investigated yet. The aim of the present study was to use staining with monoclonal antibodies to different cell surface molecules and flow cytometry to analyse the expression pattern of cell markers on camel blood eosinophils.

In the present study, the comparison between camel blood eosinophils and neutrophils revealed significantly ($p < 0.05$) higher side light scatter (SSC) and lower forward light scatter (FSC) for eosinophils than neutrophils (Fig 1A and B). In addition, camel eosinophils showed a significantly ($p < 0.05$) higher autofluorescence in the green fluorescence channel (FL-1) when compared with neutrophils (Fig 1A and Fig 2). These findings indicate, that camel blood eosinophils can be identified as SSChigh/FSClow/FL-1high granulocytes, which is similar to the phenotype of human (Gorczyca *et al,* 2011) and bovine (Pelan-Mattocks *et al,* 2001) eosinophils.

CD45 is a phosphotyrosine phosphatase expressed on the surface of all leukocytes and is known to play a critical role in the regulation of both lymphoid and myeloid cell function (Liles *et al,* 1995). CD45 cross-linking on human eosinophils significantly increased ROS production response to stimulation with GM-CSF- and TNF-alpha (Liles *et al,* 1995). CD44 is a type I transmembrane glycoprotein that is expressed by most cell types, including leukocytes, and is the major cell surface receptor for hyaluronan (HA) (Wang *et al,* 2002; Senbanjo and Chellaiah, 2017). CD44 plays a central role as an essential adhesion molecule involved in the migration of human blood eosinophils to the respiratory tract in bronchial asthma (Sano *et al,* 1997). CD11a dimerises with CD18 to form the adhesion molecule lymphocyte function antigen-1 (LFA-1) expressed on all leukocytes (Roos and Law, 2001; van de Vijver *et al,* 2012). In the present study, camel eosinophils showed a significantly ($p < 0.05$) higher abundance of the pan leukocyte marker CD45 than neutrophils (Fig 2). In addition, camel eosinophils expressed the cell adhesion molecules CD44 and CD11a in a higher density than neutrophils (Fig 2). The functional importance of different expression densities of CD45, CD44, and CD11a on camel eosinophils and neutrophils needs further investigation.

CD172a, which is known as signal-regulatory protein alpha (SIRPa), is glycosylated cell surface receptor expressed on myeloid cells and functions as a regulatory receptor that inhibits cell signaling

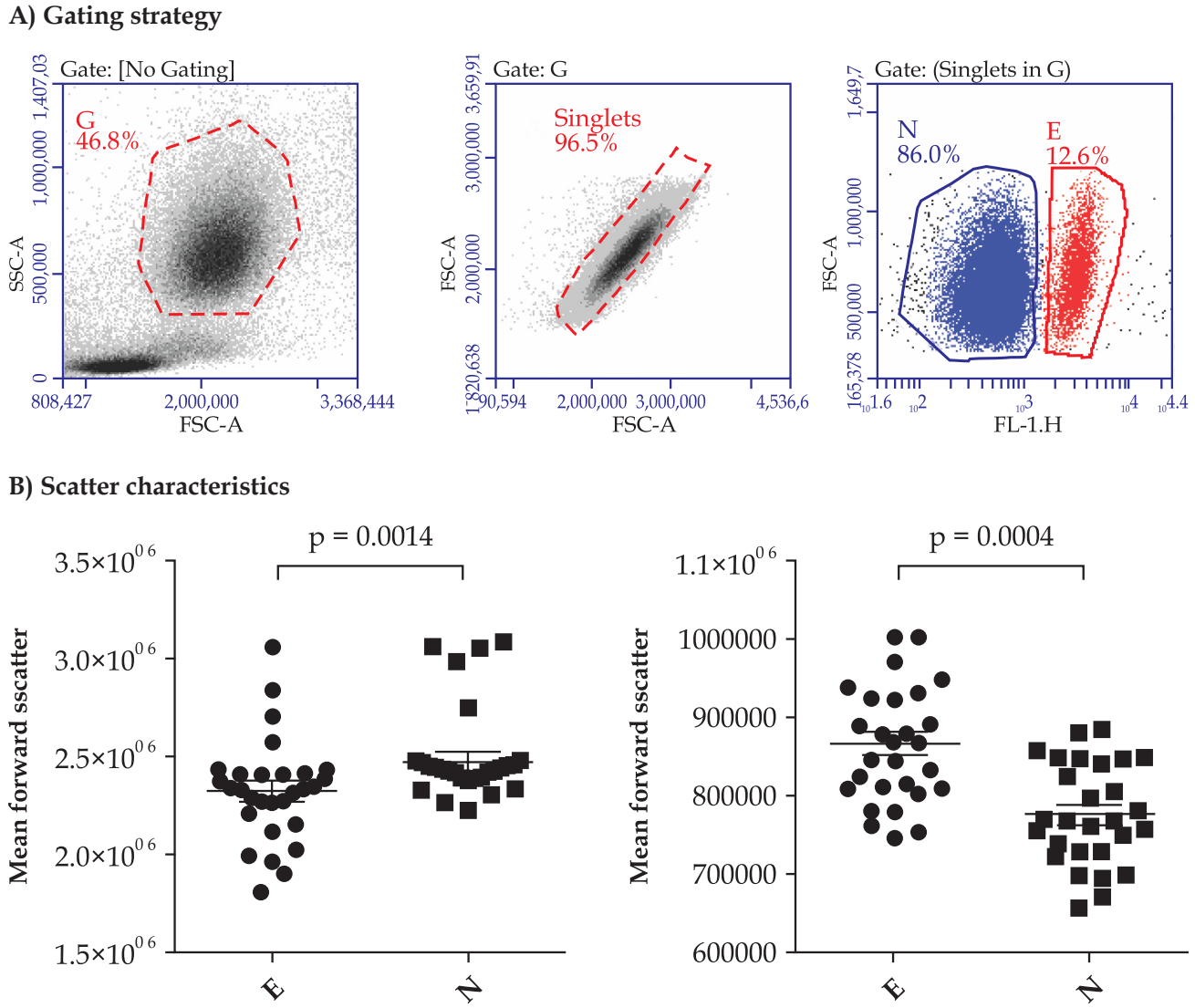

Fig 1. **A)** Gating strategy for camel blood eosinophils. In a forward light scatter (FSC-A) / side light scatter (SSC-A) dot plot, a gate was set on granulocytes (G) a according to their scatter characteristics. Duplets were excluded from the analysis by setting a gate on single cells in a FSC-A against FAC-H dot plot. In a SSC-A/FL-1 dot plot, eosinophils were identified within the granulocytes population by their higher green fluorescence than neutrophils in the Fl-1 channel. **B)** The mean FSC and SSC values for eosinophils and neutrophils were calculated and presented as means ± SEM.

(Hussen *et al,* 2013). In the present study, camel eosinophils showed a significantly lower abundance of CD172a when compared to neutrophils (Fig 2).

CD14 is a membrane protein which functions together with toll-like receptor 4 (TLR-4) as a bacterial pattern recognition receptor responsible for binding lipopolysaccharide (LPS) in the cell wall of gram-negative bacteria (Payne *et al,* 1993). Although it was mainly found on monocytes, camel neutrophils also show a low expression level of CD14 (Hussen, 2018). In the current work, the LPS receptor CD14 was expressed in a significantly lower intensity on camel eosinophils than neutrophils (Fig 2). Whether this can be linked to more involvement of neutrophils in sensing of LPS from gram-negative bacteria, still to be investigated.

Similar to neutrophils, camel eosinophils were found negative for the surface molecules CD163 (data not shown).

Conclusions

Based on their light scatter charecterestics and their green autofluorescence, camel eosinophilic granulocytes were identified as SSChigh/FSClow/Fl-1high cells within the granulocyte population. In comparision to neutrophilic granulocytes, camel eosinophils showed higher abundance of the cell surface molecules CD45, CD44, and CD11a but lower

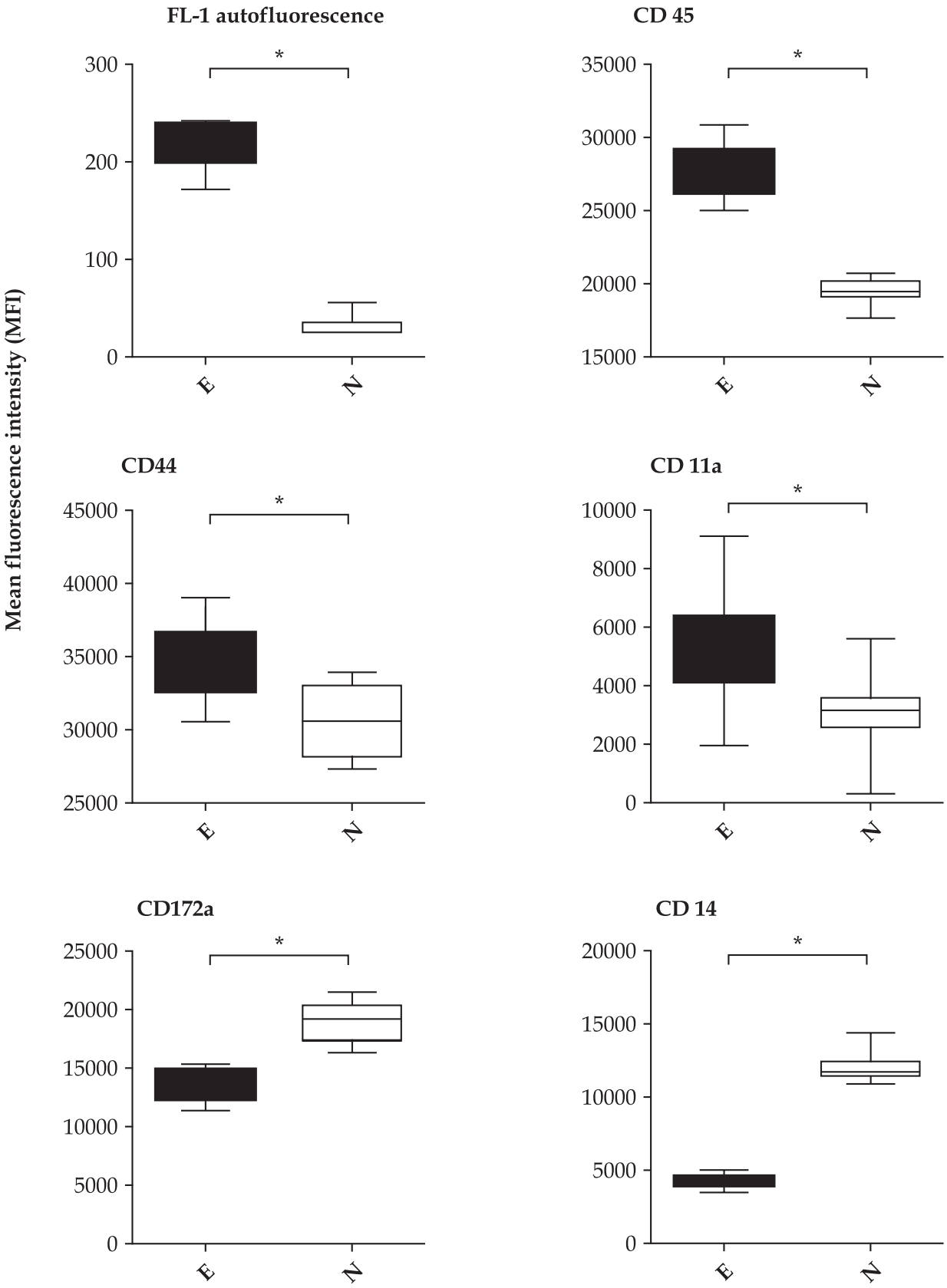

Fig 2. After gating on eosinophils (E) and neutrophils (N), the mean autofluorescence intensity in Fl-1 and the expression densities (MFI) of the cell surface molecules, CD45, CD44, CD11a, CD14, and CD172a were calculated and presented for eosinophils and neutrophils as means ± SEM. Statistical significance is indicated as * ($P < 0.05$.)

abundance of the cell markers CD172a and CD14. Further investigations are needed to analyse the impact of the differencies in immunophenotype between eosinophils and neutrophils on their functions in camel immunology.

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