DOI : 10.5958/2277-8934.2021.00023.0 *Vol 28 No 2, p 137-141*

PLASMA FROM *Escherichia coli* AND *Staphylococcus aureus* STIMULATED BLOOD DIFFERENTLY MODULATES THE EXPRESSION OF MONOCYTIC MARKERS ON CAMEL LEUKOCYTES

Naser Abdallah Al Humam
Department of Microbiology, College of Veterinary Medicine, King Faisal University, Al-Ahsa, Saudi Arabia

ABSTRACT

Escherichia coli (*E. coli*) and *Staphylococcus aureus* (*S. aureus*) are two bacterial pathogens responsible for different infectious diseases in the dromedary camel with different disease outcomes. In other species, different host-pathogen interaction mechanisms have been reported for *E. coli* and *S. aureus*. To investigate the modulatory effects of the two pathogen species on the camel innate immune system, the present study used plasma samples collected from *E. coli* and *S. aureus* blood cultures for stimulation of camel leukocytes *in vitro*. Using labeling of cell markers with monoclonal antibodies and flow cytometry, the changes in the expression of several cell markers on monocytes and neutrophilic granulocytes were identified. Plasma from either *E. coli* or *S. aureus* blood cultures resulted in a significant decrease in the expression level of CD14 on blood monocytes, the decrease was, however, significantly stronger for plasma from *E. coli* than *S. aureus* blood culture. In addition, only plasma from *E. coli* blood culture was able to reduce the expression of CD14 on stimulated granulocytes. This may represent an immune evasion mechanism of *E. coli* from the CD14-mediated innate recognition of gram-negative bacteria by camel monocytes and neutrophils. No changes were observed in the expression of CD163, MHCII or CD44 on neutrophils stimulated with plasma from either of the *E. coli* or *S. aureus* blood cultures. The different effects of plasma collected from *E. coli* stimulated blood and *S. aureus* stimulated blood on monocytes and neutrophils indicates a bacterial-species-specific modulating effect on camel monocytes and neutrophils.

Key words: Camel, bacterial blood culture, blood plasma, *Escherichia coli*, monocytes, neutrophils, *Staphylococcus aureus*

Escherichia coli (*E. coli*) and *Staphylococcus aureus* (*S. aureus*) are major causative agents of different infectious diseases in the dromedary camel including metritis, mastitis in adults and respiratory and intestinal infections in newborns (Tibary *et al,* 2006; El Tigani-Asil *et al,* 2020). While *S. aureus* is mainly responsible for subclinical infections of the udder (Keefe, 1997; Bannerman *et al,* 2004; Keefe, 2012; Gunther *et al,* 2016), infection with *E. coli* results mostly in severe acute inflammatory disease with clinical signs (Bannerman *et al,* 2004).

Monocytes and neutrophilic granulocytes are innate immune cells with an essential role during the innate immune response to bacterial pathogens (Jakubzick *et al,* 2017). For their antimicrobial effects, monocytes and neutrophils are equipped with several cell surface molecules, which play key roles in pathogen detection, phagocytosis, and elimination (Ziegler-Heitbrock, 2000; Auffray *et al,* 2007). The cell surface molecules CD14, CD163, and MHCII have been proven as reliable markers of the innate function of several myeloid cells (Schwartz and Svistelnik, 2012; Thawer *et al,* 2013; Hussen *et al,* 2014; Hussen and Schuberth, 2017). Major histocompatibility (MHC) class II molecules are antigen receptors expressed on blood monocytes and B cells, and present antigens to T helper cells (Abeles *et al,* 2012). CD163 is a scavenger receptor for haptoglobin–haemoglobin complexes that is mainly expressed on monocytes and macrophages.

In other species, different host-pathogen interaction mechanisms have been reported for *E. coli* and *S. aureus*. The aim of the current study was to investigate the modulatory effects of plasma samples collected from *E. coli* and *S. aureus* blood cultures on the camel innate immune cells monocytes and neutrophils upon *in vitro* stimulation.

Materials and Methods

Blood samples were collected from healthy camels (n = 10; *Camelus dromedarius*) by venipuncture

SEND REPRINT REQUEST TO NASER ABDALLAH AL HUMAM email: nalhumam@kfu.edu.sa

of the vena jugularis externa into vacutainer tubes containing EDTA (Becton Dickinson, Heidelberg, Germany). For the preparation of blood plasma, collected blood samples (n = 4) were stimulated with *E. coli* or *S. aureus* (two mastitis isolates) according to a previously established method (Hussen *et al,* 2013). Whole camel blood (2 ml) was diluted with 1.8 ml cell culture medium (RPMI-1640, Sigma-Aldrich, Deisenhofen, Germany) in sterile 15 ml tubes (BD Biosciences, San Jose, California, USA). Live bacterial suspension (0.2 ml; 10^7 bacteria/ml) was added to the diluted blood and the mixture was then incubated for 12 h at 37°C. A control tube containing 2 ml blood and 2 ml medium without bacteria was also included. After incubation, the tubes were then put into icy water and immediately centrifuged at 4°C for 10 min at 1000xg to collect the supernatant plasma. Collected stimulated and unstimulated plasma samples from all animals were pooled together, and the pooled plasma was used for further stimulation of camel leukocytes. 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).

Separation of camel leukocytes and stimulation with plasma

Separation of camel leukocytes (n = 6 animals) was done after hypotonic lysis of blood erythrocytes as previously described (Hussen *et al,* 2017). Briefly, unstimulated and stimulated blood samples suspended in PBS were centrifuged at 4°C for 10 min at 1000xg and the cell pellet was suspended in distilled water for 20 sec and double concentrated PBS was added to restore tonicity. This was repeated until complete erythrolysis. Separated cells were finally suspended in RPMI medium (1×10^6/ ml). Separated leukocytes were stimulated in RPMI medium with plasma from control (non-stimulated blood samples), plasma from *E. coli* blood culture, plasma from *S. aureus* blood culture, or the cells were left unstimulated in RPMI medium (without plasma).

Monoclonal antibodies

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

Membrane immunofluorescence and flow cytometry

The expression densities of different monocytic cell surface molecules were evaluated by flow cytometry after membrane immunofluorescence (Eger *et al,* 2015). Stimulated and unstimulated leukocytes (2×10^5) were incubated with unlabeled primary monoclonal antibodies (mAbs) specific for the cell markers CD14, MHCII, CD163, and CD44 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 100 000 total leukocytes were collected and analysed with the CFlow Software, Version 1.0.264.21 (Fig 1A and Fig 2A).

Table 1. List of antibodies.

Antigen	Antibody clone	Label	Source	Isotype
CD14	TÜK4	-	WSU	mIgG1
MHCII	TH81A5	-	Kingfisher	mIgG2a
CD163	LND68A	-	Kingfisher	mIgG1
CD44	LT41A	-	WSU	mIgG2a
mIgG2a	polyclonal	PE	Invitrogen	gIgG
mIgG1	polyclonal	FITC	Invitrogen	gIgG

Ig: Immunoglobulin; m: mouse; g: goat, MHCII: Major Histocompatibility Complex class II, FITC: Fluorescein isothiocyanate, PE: Phycoerythrin.

Statistical Analyses

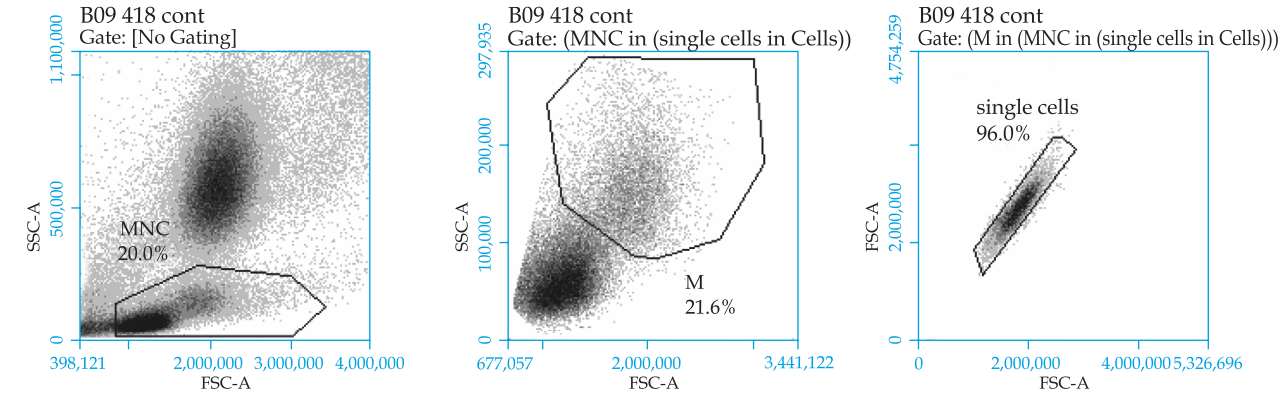
Statistical analysis was carried out using the software Prism (GraphPad software version 5). Results are expressed as mean ± S.E. (SEM). Differences between means were tested with one-factorial analysis of variance (ANOVA). Results were considered statistically significant at a p-value of less than 0.05.

Results and Discussion

Monocytes and neutrophils are equipped with several cell surface molecules, which play key roles in pathogen detection, phagocytosis, and elimination (Ziegler-Heitbrock, 2000; Auffray *et al,* 2007). CD14 is a membrane protein mainly expressed on monocytes, and it serves 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). In addition, camel neutrophils show a low expression level of the LPS co-receptor CD14, which is similar to bovine neutrophils (Sohn *et al,* 2007), suggesting a role for neutrophils in the sensing of gram-negative bacteria (Hussen, 2018).

In the present study, stimulation with plasma samples collected from either *E. coli* or *S. aureus*

A) Gating strategy for blood monocytes



B) Expression density of cell surface molecules on monocytes

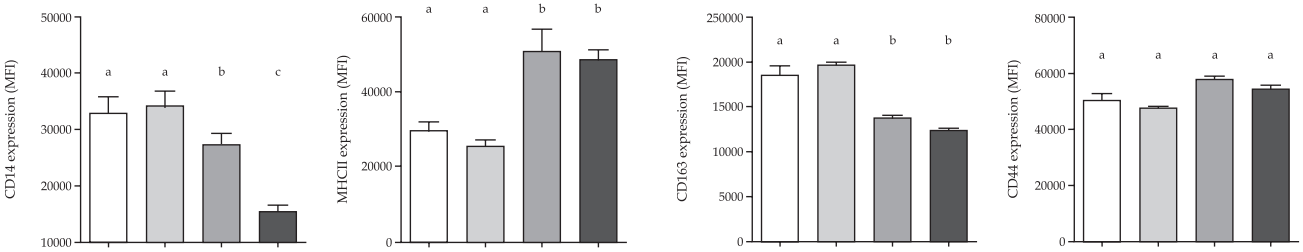


Fig 1. **A)** Gating strategy for camel blood monocytes. In a forward scatter (FSC-A) / side scatter (SSC-A) dot plot, a gate was set on mononuclear cells (MNC) a according to their scatter characteristics. After gating on MNC, monocytes were identified in a separate FSC-A/SSC-A dot plot according to their FSC and SSC properties. Duplets were excluded from the analysis by setting a gate on single cells in a FSC-A against FAC-H dot plot. **B)** The expression densities of different cell markers on stimulated and non-stimulated monocytes. The mean fluorescence intensity of the cell surface molecules, CD14, MHCII, CD163, and CD44 were calculated and presented for unstimulated monocytes and monocytes stimulated with plasma as means ± SEM. Different lowercase superscript letters indicate statistical significance ($P < 0.05$).

blood cultures resulted in a significant decrease in the expression level of CD14 on blood monocytes (Fig 1B). This decrease in CD14 on monocytes was, however, significantly ($p < 0.05$) stronger for monocytes stimulated with plasma from *E. coli* than *S. aureus* blood culture (Fig 1B). For stimulated granulocytes, only plasma from *E. coli* blood culture was able to reduce the expression of CD14 on stimulated granulocytes in comparison to cells stimulated with plasma from *S. aureus* blood culture of unstimulated blood (Fig 2B). This may indicate a suppressive effect of plasma collected from *E. coli* blood culture on the CD14-mediated innate recognition function of monocytes and neutrophils toward gram-negative bacterial pathogens.

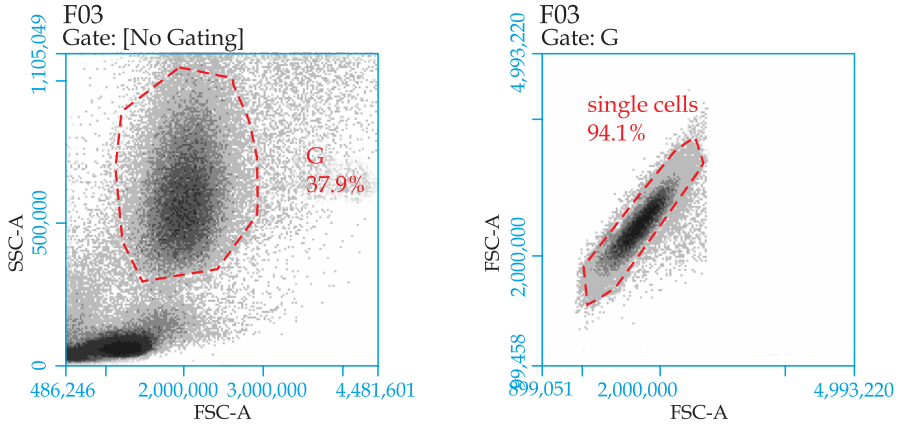
The expression levels of MHCII and CD163 are widely accepted as markers for pro- (macrophages subtype 1; M1) and anti-inflammatory (macrophage subtype 2; M2) functional subtypes of macrophages, respectively (Hu *et al,* 2017). In the current study, plasma samples collected from either *E. coli* or *S. aureus* blood cultures induced a significantly ($p < 0.05$) higher abundance of MHCII and a significantly ($p < 0.05$) lower abundance of CD163 on camel monocytes (Fig 1B). The increased expression of the M1 marker MHCII together with the reduced expression of the M2 marker CD163 indicate a pro-inflammatory phenotype of monocytes stimulated with plasma samples collected from the two bacterial blood cultures.

In contrast to the stimulation-induced change in the phenotype of monocytes, there were no changes in the expression of the cell markers MHCII, CD163, or CD44 on neutrophils upon incubation with plasma from the bacterial blood cultures (Fig 2B). This indicates different modulatory effects of the two pathogen species on monocytes and neutrophils. Whether this is due to the existence of different immune mediators in the plasma samples collected from stimulated blood, still need to be investigated.

Conclusions

The enhanced expression of MHCII molecules together with the reduced expression of CD163

A) Gating strategy for blood granulocytes



B) Expression density of cell surface molecules on granulocytes

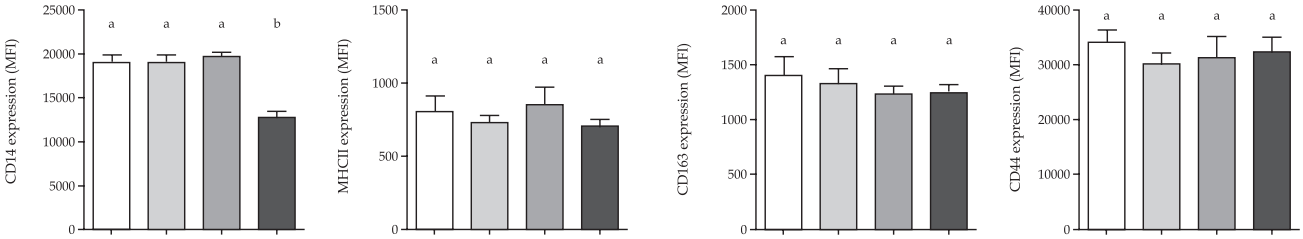


Fig 2. **A)** Gating strategy for camel blood granulocytes. In a forward scatter (FSC-A) / side scatter (SSC-A) dot plot, a gate was set on granulocytes (G) according to their scatter characteristics. After gating on granulocytes, duplets were excluded from the analysis by setting a gate on single cells in a FSC-A against FAC-H dot plot. **B)** The expression densities of different cell markers on stimulated and non-stimulated granulocytes. The mean fluorescence intensity of the cell surface molecules, CD14, MHCII, CD163, and CD44 were calculated and presented for unstimulated granulocytes and granulocytes stimulated with plasma as means ± SEM. Different lowercase superscript letters indicate statistical significance ($P < 0.05$).

molecules on camel monocytes stimulated with plasma from bacteria-stimulated blood indicates the shift of monocytes toward a pro-inflammatory phenotype. Plasma from *E. coli* stimulated blood resulted in a stronger decrease in CD14 expression on monocytes and a significant lower CD14 abundance on neutrophils, when compared with plasma from *S. aureus* blood culture. This may represent an immune evasion mechanism of *E. coli* against the CD14-mediated innate recognition of gram-negative bacteria by monocytes and neutrophils. The different effects of plasma collected from *E. coli* and *S. aureus* blood cultures on monocytes and neutrophils indicates a bacterial-species-specific modulating effect on camel monocytes and neutrophils.

Acknowledgements

The authors acknowledge the Deanship of Scientific Research at King Faisal University, Saudi Arabia for the financial support of this work under Nasher Track (grant Nr. 206132).

References

Abeles RD, McPhail MJ, Sowter D, Antoniades CG, Vergis N, Vijay GKM, Xystrakis E, Khamri W, Shawcross DL, Ma Y, Wendon JA and Vergani D (2012). CD14, CD16 and HLA-DR reliably identifies human monocytes and their subsets in the context of pathologically reduced HLA-DR expression by CD14(hi) /CD16(neg) monocytes: Expansion of CD14(hi) /CD16(pos) and contraction of CD14(lo) /CD16(pos) monocytes in acute liver failure. Cytometry. Part A : The Journal of the International Society for Analytical Cytology 81(10)823-834. https://doi.org/10.1002/cyto.a.22104

Auffray C, Fogg D, Garfa M, Elain G, Join-Lambert O, Kayal S, Sarnacki S, Cumano A, Lauvau G and Geissmann F (2007). Monitoring of blood vessels and tissues by a population of monocytes with patrolling behaviour. Science 3175838:666-70.

Bannerman DD, Paape MJ, Hare WR and Hope JC(2004). Characterisation of the bovine innate immune response to intramammary infection with *Klebsiella pneumoniae*. Journal of Dairy Science 878:2420-32.

Eger M, Hussen J, Drong C, Meyer U, von Soosten D, Frahm J, Daenicke S, Breves G and Schuberth HJ (2015). Impacts of parturition and body condition score on glucose uptake capacity of bovine monocyte subsets.

Veterinary Immunology and Immunopathology 1661-2:33-42.

El Tigani Ahmed El Tigani-Asil, Ghada Elderdiri Abdelwahab, Jayamohanan Thikkal Veedu Payyan Veedu, Abdelmalik Ibrahim Khalafalla, Zayed Saud Abdullah Mohamed, Hassan Zackaria Ali Ishag, Asma Abdi Mohamed Shah, Mohamed Ali Abdulla Alhosani, and Salama Suhail Mohammed Al Muhairi (2020). Gangrenous mastitis in dromedary camels in UAE caused by *Streptococcus agalactiae*. BMC Veterinary Research 161:174.

Gunther J, Koy M, Berthold A, Schuberth HJ and Seyfert HM (2016). Comparison of the pathogen species-specific immune response in udder derived cell types and their models. Veterinary Research 47:22.

Hu JM, Liu K, Liu JH, Jiang XL, Wang XL, Chen YZ, Li SG, Zou H, Pang LJ, Liu CX, Cui XB, Yang L, Zhao J, Shen XH, Jiang JF, Liang WH, Yuan XL and Li F (2017). CD163 as a marker of M2 macrophage, contribute to predicte aggressiveness and prognosis of Kazakh esophageal squamous cell carcinoma. Oncotarget 813:21526-38.

Hussen J (2018). Flow cytometric analysis of phenotype and composition of peripheral blood leukocytes in young and old dromedary camels (*Camelus dromedarius*). Journal of Camel Practice And Research 25(1):29-36.

Hussen J, Düvel A, Sandra O, Smith D, Sheldon IM, Zieger P, Schuberth H-J (2013). Phenotypic and functional heterogeneity of bovine blood monocytes. PLoS One 8(8):e71502. PLoS One 8(8):e71502.

Hussen J, Frank C, Duvel A, Koy M and Schuberth HJ (2014). The chemokine CCL5 induces selective migration of bovine classical monocytes and drives their differentiation into LPS-hyporesponsive macrophages *in vitro*. Developmental and Comparative Immunology 472:169-77.

Hussen J and Schuberth HJ (2017). Heterogeneity of bovine peripheral blood monocytes. Frontiers in Immunology 8:1875.

Hussen J, Shawaf T, Al-Herz AI, Alturaifi HR and Alluwaimi AM (2017). Reactivity of commercially available monoclonal antibodies to human CD antigens with peripheral blood leucocytes of dromedary camels (*Camelus dromedarius*). Open Veterinary Journal 72:150-53.

Jakubzick CV, Randolph GJ and Henson PM (2017). Monocyte differentiation and antigen-presenting functions. Nature Reviews Immunology 176:349-62.

Keefe G (2012). Update on control of *Staphylococcus aureus* and *Streptococcus agalactiae* for management of mastitis. Veterinary Clinics of North America Food Animal Practice 282:203-16.

Keefe GP (1997). *Streptococcus agalactiae* mastitis: a review. Canadian Veterinary Journal 387:429-37.

Payne NR, Frestedt J, Hunkeler N and Gehrzv R (1993). Cell-surface expression of immunoglobulin G receptors on the polymorphonuclear leukocytes and monocytes of extremely premature infants. Pediatric Research 33(5):452-7.

Schwartz Y and Svistelnik AV (2012). Functional phenotypes of macrophages and the M1-M2 polarisation concept. Part I. Proinflammatory phenotype. Biochemistry (Mosc) 773:246-60.

Sohn EJ, Paape MJ, Bannerman DD, Connor EE, Fetterer RH and Peters RR (2007). Shedding of sCD14 by bovine neutrophils following activation with bacterial lipopolysaccharide results in down-regulation of IL-8. Veterinary Research 381:95-108.

Thawer SG, Mawhinney L, Chadwick K, de Chickera SN, Weaver LC, Brown A and Dekaban GA (2013). Temporal changes in monocyte and macrophage subsets and microglial macrophages following spinal cord injury in the Lys-Egfp-ki mouse model. Journal of Neuroimmunology 2611-2:7-20.

Tibary A, Fite C, Anouassi A and Sghiri A (2006). Infectious causes of reproductive loss in camelids. Theriogenology 663: 633-47.

Ziegler-Heitbrock HsW (2000). Definition of human blood monocytes. Journal of Leukocyte Biology 675:603-6.