Open Veterinary Journal, (2017), Vol. 7(2): 150-156
ISSN: 2226-4485 (Print)
ISSN: 2218-6050 (Online)
Original Article
DOI: http://dx.doi.org/10.4314/ovj.v7i2.12

Submitted: 01/02/2017 Accepted: 15/05/2017 Published: 28/05/2017

Reactivity of commercially available monoclonal antibodies to human CD antigens with peripheral blood leucocytes of dromedary camels (*Camelus dromedarius*)

Jamal Hussen[1,*], Turke Shawaf[2], Abdulkareem Imran Al-herz[3], Hussain R. Alturaifi[3] and Ahmed M. Alluwaimi[1]

[1]*Department of Microbiology and Parasitology, College of Veterinary Medicine, King Faisal University, Al Ahsaa, Saudi Arabia*
[2]*Department of Clinical Studies, College of Veterinary Medicine, King Faisal University, Al Ahsaa, Saudi Arabia*
[3]*Immunology Unit, Diagnostic Laboratory and Blood Bank, King Fahad Hospital Hufof, Al Ahsaa, Saudi Arabia*

Abstract
Monoclonal antibodies (mAbs) to cell surface molecules have been proven as a key tool for phenotypic and functional characterization of the cellular immune response. One of the major difficulties in studying camel cellular immunity consists in the lack of mAbs that dtect their leukocyte differentiation antigens. In the present study two-parameter flow cytometry was used to screen existing commercially available mAbs to human leukocyte antigens and major histocompatibility molecules (MHC) for their reactivity with camel leukocytes. The comparison of patterns of reactivity obtained after labelling human and camel leukocytes have shown that mAbs specific to human cluster of differentiation (CD) 18, CD11a, CD11b and CD14 are predicted to be cross-reactive with homologous camel antigens.
Keywords: Antibodies, Cross-reactivity, Dromedary camel, Flow cytometry.

Introduction

The immune system consists of a complex network of cellular and non-cellular components, which interact with each other to protect the animal against invading pathogens like bacteria, fungi, parasites and viruses. In comparison to several other veterinary species like cattle (Hussen *et al*., 2013; Duvel *et al*., 2014), pigs (Gerner *et al*., 2015), sheep (Hopkins *et al*., 1993) and horses (Lunn *et al*., 1998), the immune system of camels remains to a great extent poorly studied. Although considerable progress has been made in the characterization of camel immunoglobulins (Hamers-Casterman *et al*., 1993; Muyldermans, 2013), few data are available on the cellular immunity of camels in health or disease (Zidan *et al*., 2000a,b; Al-Mohammed Salem *et al*., 2012).

Monoclonal antibodies (mAbs) to leukocyte antigens are highly important tools for phenotypic and functional analysis of cellular immunology. The lack of mAbs that define camel immune cells represents one of the major difficulties in studying the camel cellular immune response.

As the production of mAbs is very costly, attempts are made to study the cross-reactivity of commercially available mAbs to leukocyte antigens of one species with leukocytes of other species.

Comparative studies with mAb to leukocyte antigens of ruminants, swine, horses, and dogs have shown that the pattern of expression of many molecules is conserved cross species (Davis *et al*., 1995). These findings suggests that it would be useful to use comparative studies for the identification of mAbs that recognize conserved epitopes on leukocyte differentiation antigens in poorly studied species.

The identification of cross-reactive mAbs could reduce the need to develop reagents for some important molecules and would provide an opportunity to compare the immune systems of camel with that of other species.

The objective of the present study was to screen existing mAbs to human leukocyte antigens and major histocompatibility molecules (MHC) for their reactivity with camel leukocytes. This would help in identifying mAbs that could be used to study the immune response of camels to infectious pathogens and as well as their response to vaccination.

Materials and Methods

Animals

Blood was collected from four camels (*Camelus dromedaries;* males aged between 6 and 8 years) at Omran slaughterhouse, Al Ahsaa, Eastern Province, Saudi Arabia.

The camels included in the study were apparently healthy and had no vaccination history. Blood was obtained by venepuncture of the vena jugularis externa into vacutainer tubes containing EDTA (Becton Dickinson, Heidelberg, Germany).

Mononuclear cells and whole leukocytes Separation

Separation of camel mononuclear cells (PBMC) was performed according to a method used for separating

***Corresponding Author:** Dr. Jamal Hussen. Department of Microbiology and Parasitology, College of Veterinary Medicine, King Faisal University, Al Ahsaa, Saudi Arabia. *Tel*.: +966135896626; *Fax*: +966135816345. E-mail: jhussen@kfu.edu.sa

bovine blood cells with modification (Hussen *et al*., 2016). Blood was layered on Ficoll-Isopaque (Sigma-Aldrich, Germany) and centrifuged at 10°C for 30 min at 3000 rpm. The interphase containing PBMC was washed 3 times in PBS (2000, 1500 and 1000 rpm) and finally suspended in MIF buffer (PBS containing bovine serum albumin (5 g/L) and NaN_3 (0.1 g/L)). For the separation of human PBMC, human blood was layered on Ficoll-Isopaque and centrifuged at 10°C for 30 min at 3000 rpm. The interphase containing PBMC was washed 3 times in PBS (2000, 1500 and 1000 rpm) and finally suspended in MIF buffer. Whole camel and human leukocytes were separated by hypotonic lysis of erythrocytes. 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 at 5 x 106 cells/ml. Cell purity of separated PBMC and leukocytes was assessed by flow cytometry according to their forward scatter (FCS) and sideward scatter (SSC) properties and always exceeded 90%. The mean viability of separated cells, as determined by exclusion of propidium iodide (2 µg/ml, Calbiochem, Germany) ranged 92% ± 4% (Fig. 1).

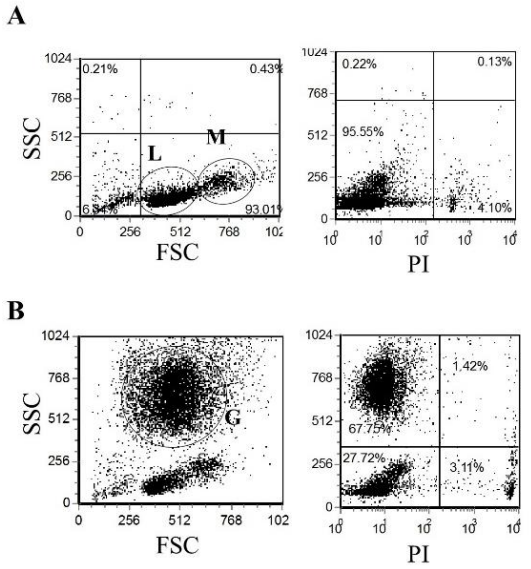

Fig. 1. Separation of camel blood PBMC and leukocytes and gating strategies. Camel PBMC were isolated by density gradient separation using Ficoll-Isopaque **(A)**. Whole camel leukocytes were separated by hypotonic lysis of erythrocytes **(B)**. Cell purity and viability of separated PBMC and leukocytes was assessed by flow cytometry according to their forward scatter (FCS) and sideward scatter (SSC) properties of the cells. The mean viability of separated cells was determined by exclusion of propidium iodide (PI). Gates were placed on granulocytes (G), monocytes (M) and lymphocytes (L) (as displayed in dot plot profile, side light scatter vs forward light scatter).

Monoclonal antibodies

The full list of mAbs (52 commercially available antibodies) used in this study are shown in Table 1.

Table 1. List of anti-human monoclonal antibodies.

Antigen	Antibody clone	Source	Isotype
CD2	S5.2	BD	mIgG2a
CD3	SK7 (Leu-4)	BD	mIgG1
CD4	SK3	BD	mIgG1
CD5	L17F12	BD	mIgG2a
CD7	M-T701	BD	mIgG1
CD7	4H9	BD	mIgG2a
CD8	SK1	BD	mIgG1
CD9	M-L13	BD	mIgG1
CD10	HI10a	BD	mIgG1
CD11a	G43-25B	BD	mIgG2a
CD11b	ICRF44	BD	mIgG1
CD11c	S-HCL-3	BD	mIgG2b
CD11c	KB90	Dako	mIgG1
CD13	L138	BD	mIgG1
CD14	M5E2	BD	mIgG2a
CD14	TÜK4	Biorad	mIgG2a
CD14	MφP9	BD	mIgG2b
CD15	MMA	BD	mIgM
CD16	B73.1	BD	mIgG1
CD16	KD1	BD	mIgG2a
CD18	42557	BD	mIgG1
CD19	4G7	BD	mIgG1
CD19	SJ25C1	BD	mIgG1
CD20	L27	BD	mIgG1
CD20	B9E9	Coulter	mIgG2a
CD20	2H7	Abcam	mIgG2b
CD22	S-HCL-1	BD	mIgG2b
CD33	P67.6	BD	mIgG1
CD38	HB7	BD	mIgG1
CD38	HIT2	BD	mIgG1
CD45	2D1	BD	mIgG1
CD55 (DAF)	JS11KSC2.3	Coulter	mIgG1
CD56	NCAM16.2	BD	mIgG2b
CD58 (LFA-3)	1C3	BD	mIgG2a
CD62L	DREG-56	BD	mIgG1
CD64	42379	BD	mIgG1
CD79a	HM47	Coulter	mIgG1
CD79b	CB3.1	BD	mIgG1
CD95	DX2	Dako	mIgG1
CD99	TÜ12	BD	mIgG2a
CD126	M5	BD	mIgG1
CD182	6C6	BD	mIgG1
TCR-αβ	WT31	BD	mIgG1
TCR-γ/δ	11F2	BD	mIgG1
IgM	G20-127	BD	mIgG1
Ig-lambda chain	1-155-2	BD	mIgG1
Ig-Kappa chain	TB28-2	BD	mIgG1
MHC-II	L243	BD	mIgG2a
MHC-II	G46.2.6	BD	mIgG1
bcl-2	Bcl-2/100	BD	mIgG1
Kappa chain	TB28-2	BD	mIgG1
Lambda chain	1-155-2	BD	mIgG1

Ig: Immunoglobulin; m: mouse; MHC-II: Major Histocompatibility Complex class II.

Immunofluorescence and flow cytometry

Camel or human cells (PBMC or leucocytes; 4 x 10^5) were incubated with mAbs specific for human CD antigens (Table 1) in PBS containing bovine serum albumin (5 g/l) and NaN_3 (0.1 g/l). After 30 minutes incubation (4°C), cells were washed twice and analyzed on the flow cytometer. A Becton Dickinson FACSCalibur equipped with Cell Quest software (FACSCalibur (Becton Dickinson Biosciences, San Jose, California, USA) was used to collect the data. At least 100 000 cells were collected and analyzed with the FCS Express software Version 3 (De Novo Software, Thornton, Ontario).

In order to exclude signals due to non-specific binding of mouse antibodies, negative isotype controls for mouse IgG1, IgG2a, IgG2b (from BD) and IgM (from Beckmann Coulter) were also included as part of the study.

Results

Flow cytometry was used to determine the reactivity of camel leukocytes with commercially available mAbs to human leukocyte markers. PBMC were isolated by density gradient centrifugation using Ficoll-Isopaque as standard method for studying the phenotype of PBMC without interfering effects of granulocytes (Fig. 1A). Whole leukocytes were separated by hypotonic lysis of erythrocytes (Fig. 1B). Separated camel or human cells were incubated with mouse mAbs specific for human leukocyte antigens or with mouse isotype control antibodies (Table 1) and were analyzed by flow cytometry. For the analysis of population-specific pattern of expression, gates were done for lymphocytes (L), monocytes (M) and granulocytes (G) (Fig.1). The profiles obtained for each mAb were then compared to the labeling pattern of reactivity obtained with human leukocytes.

Reactivity of mAbs to human leukocyte antigens with camel PBMC

The mAb 555923 (clone 6.7; from BD) is a FITC-labelled mouse antibody against human CD18. The expression pattern of CD18 on human and camel PBMC is shown in Fig.2. The mAb 555923 stained all human and camel PBMC, although the expression on lymphocytes was comparatively lower than monocytes. Also for both species, a minor subpopulation of lymphocytes remained negative for mAb 555923.

The mAb 555380 (clone G43-25B; from BD) is a PE-labelled mouse antibody directed against human CD11a. The expression pattern of CD11a on human and camel PBMC is shown in Fig.3. Although it stained all population of human leucocytes widely, mAb 555380 indicated a higher expression of CD11a on human monocytes and a subset of human lymphocytes. For camel cells, the mAb 555380 stained all cell populations weekly than human cells.

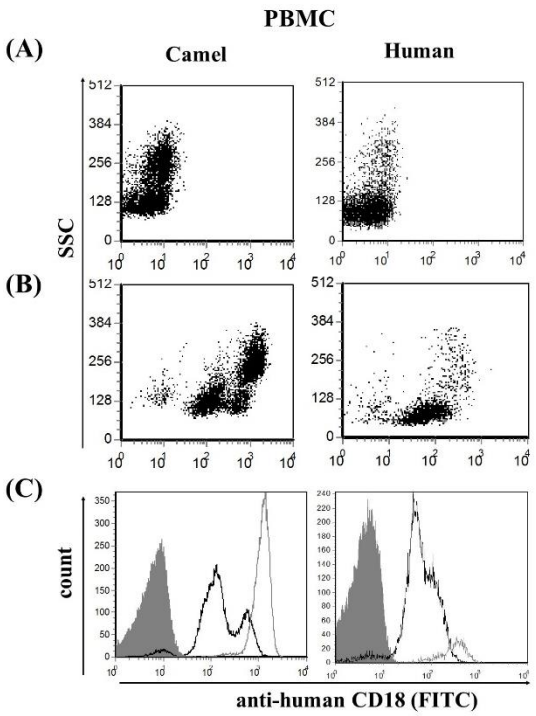

Fig. 2. Analysis of the expression pattern of CD18 on camel and human PBMC. Ficoll-separated camel or human PBMC were incubated with FITC-labelled mouse isotype control antibody **(A)** or the FITC-labelled monoclonal antibody 555923 (clone 6.7) specific for human CD18 **(B)** and analysed on the flow cytometer. The expression pattern of CD18 on camel or human PBMC was analysed by plotting SSC against CD18 expression. After gating lymphocytes and monocytes according to their forward and side scatter characteristics, the expression density of CD18 was shown in an overlapping histogram **(C)**.

However a higher expression could be seen for camel monocytes and a subset of lymphocytes.

The mAb 557743 (clone ICRF44; from BD) is a PE-Cy7-labelled mouse antibody directed against human CD11b. The expression pattern of CD11b on human and camel PBMC is shown in Fig.4. For both human and camel PBMC the mAb 557743 stained only monocytes and a minor subpopulation of lymphocytes, whereas the majority of lymphocytes was negative for this antibody.

The mAbs, 555398 (clone M5E2; from BD) and MCA1568PE (clone Tük4; from Bio-Rad) are PE-labelled mouse antibodies directed against human CD14. Both mAbs showed a similar staining pattern for human and camel PBMC. In both species, only the monocytes population was stained positively with both CD14 antibodies. The expression pattern of CD14 (data shown only for the clone M5E2) on human and camel PBMC is shown in Fig.5. The mouse mAb 347403 (clone L243; from BD) is an APC-labelled antibody directed against human HLA-DR antigen.

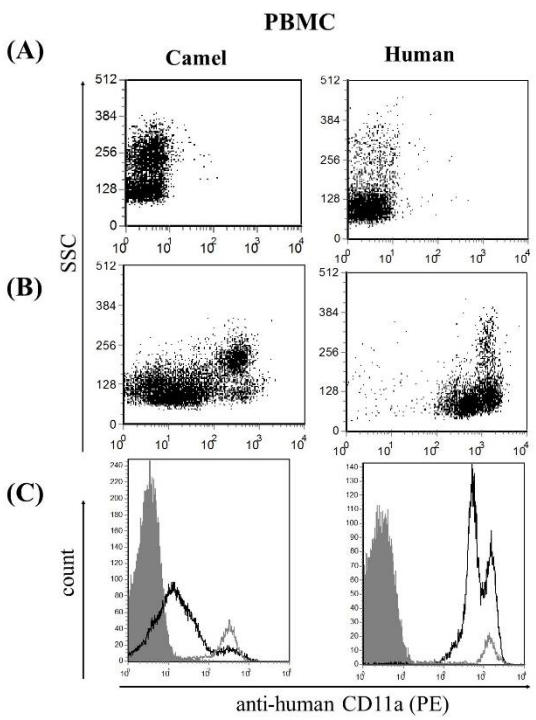

Fig. 3. Analysis of the expression pattern of CD11a on camel and human PBMC. Ficoll-separated camel or human PBMC were incubated with PE-labelled with mouse isotype control antibody (A) or the PE-labelled monoclonal antibody 555380 (clone G43-25B) specific for human CD11a (B) and analysed on the flow cytometer. The expression pattern of CD11a on camel or human PBMC was analysed by plotting SSC against CD11a expression. After gating lymphocytes and monocytes according to their forward and side scatter characteristics, the expression density of CD11a was shown in an overlapping histogram (C).

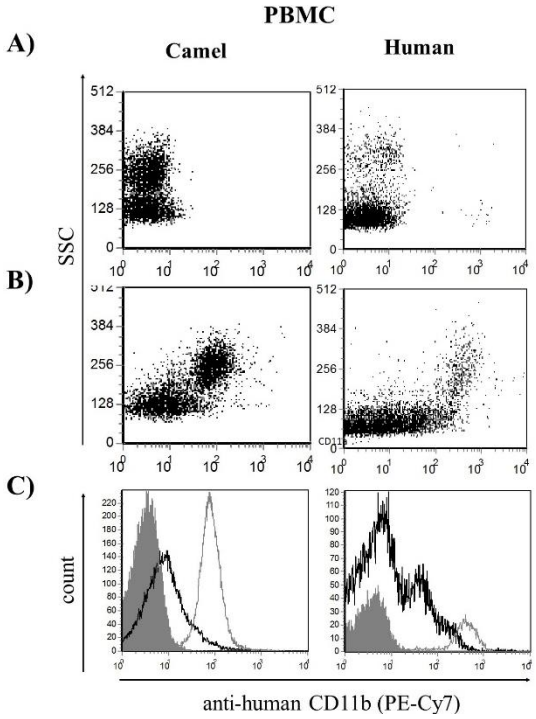

Fig. 4. Analysis of the expression pattern of CD11b on camel and human PBMC. Ficoll-separated camel or human PBMC were incubated with PE-Cy7-labelled mouse isotype control antibody (A) or the PE-Cy7-labelled monoclonal antibody 557743 (clone ICRF44) specific for human CD11b (B) and analysed on the flow cytometer. The expression pattern of CD11b on camel or human PBMC was analysed by plotting SSC against CD11b expression. After gating lymphocytes and monocytes according to their forward and side scatter characteristics, the expression density of CD11b was shown in an overlapping histogram (C).

For human PBMC the mAb 347403 stained only the monocytes population and a subpopulation of lymphocytes, which is expected to be B cells. For camel cells however, monocytes showed only week reactivity with this mAb. Only a minor subset of camel lymphocytes stained weekly positive with this mAb.

Reactivity of mAbs to human leukocyte antigens with camel granulocytes

Camel and human leukocytes were separated by hypotonic lysis of erythrocytes and were labelled with mAb to human CD antigens. Camel granulocytes showed reactivity only to three mAbs to human CD18, CD11a and CD11b (data not shown). The mAbs 555923 (FITC-labelled mouse anti-human CD18) and 555380 (PE-labelled mouse anti human CD11a) stained both human and camel granulocytes widely positive. However, a minor subpopulation of human granulocytes remained negative for the mAb 555923. Although the anti-human CD11b mAb 557743 stained both human and camel granulocytes positively, the reactivity of camel granulocytes was more weekly than that of human cells.

Only for human cells, a minor subpopulation of granulocytes remained negative for the mAb 557743.

Discussion

In comparison to the progress that has been made in the characterization of camel immunoglobulins (Hamers-Casterman *et al*., 1993; Muyldermans, 2013), few data are available on cellular immunity of camels in health or disease.

MAbs to leukocyte antigens have been considered as highly important tools for the analysis of cellular immunology in human (Maecker *et al*., 2012) as well as in different animal species (Hopkins *et al*., 1993; Lunn *et al*., 1998; Schafer and Burger, 2012; Duvel *et al*., 2014; Gerner *et al*., 2015). The lack of mAbs that define camel immune cells represents one of the major difficulties in studying the camel cellular immune response.

As the production of mAbs is very costly, the objective of the present study was to screen existing mAbs to human leukocyte antigens, immunoglobulin (Ig) chains and major MHC for their reactivity with camel leukocytes.

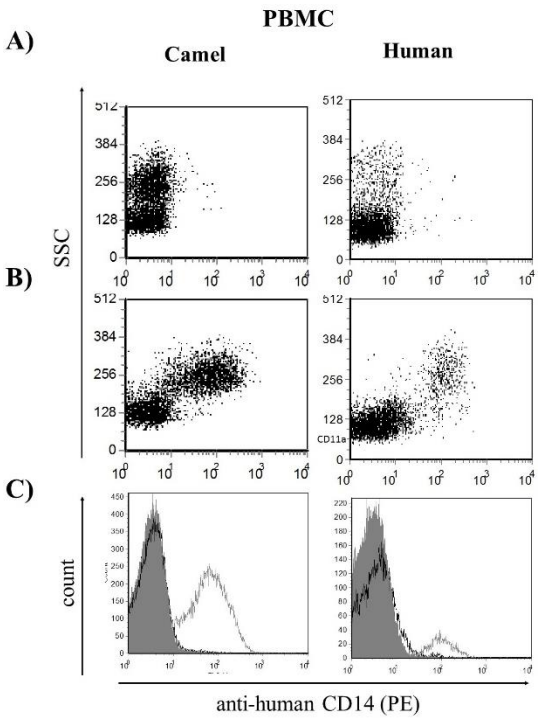

Fig. 5. Analysis of the expression pattern of CD14 on camel and human PBMC. Ficoll-separated camel or human PBMC were incubated with PE-labelled mouse isotype control antibody (A) or with the PE-labelled monoclonal antibody 555398 (clone M5E2) specific for human CD14 (B) and analysed on the flow cytometer. The expression pattern of CD14 on camel or human PBMC was analysed by plotting SSC against CD14 expression. After gating on lymphocytes or monocytes according to their forward and side scatter characteristics, the expression density of CD14 was shown in an overlapping histogram (C).

Two-parameter flow cytometry has been proven as a useful tool to study the cross-reactivity of mAbs developed against leukocyte differentiation antigens of one species with leukocyte antigens of other species (MacHugh *et al*., 1991; Naessens *et al*., 1993; Maecker *et al*., 2012). The specificity of a mAbs to a given antigen can be predicted according to the flow-cytometric pattern of expression of that molecule on labeled leukocytes (MacHugh *et al*., 1991; Naessens *et al*., 1993; Davis *et al*., 2007; Davis and Hamilton, 2008).

CD18 is the common β_2-chain (β_2-integrin) for all three forms of CD11 (a, b, c) (Harris *et al*., 2000). It has been shown that CD18 is expressed on all human leukocytes (Drbal *et al*., 2000) which is in accordance with our results of staining human PBMC and granulocytes with the mouse mAb 555923 (clone 6.7). Similar to their human counterparts, camel PBMC and granulocytes showed expressed CD18. As the patterns of reactivity for camel and human PBMC and granulocytes were identical so the homology of the proteins stained may be assumed.

Human CD11a is expressed together with CD18 as a hetero-dimer termed as leukocyte function antigen 1 (LFA-1). LFA-1 is the most important integrin expressed by all human leukocytes that regulate cell migration through binding to ICAM-1,-2 or-3 (van Kooyk and Figdor, 2000). As camel PBMC and granulocytes stained weekly than their human counterparts with the mAb 555380 (clone G43-25B) specific for human CD11a, it is likely that this mAb has a lower affinity for camel CD11a. However, the staining pattern of cell populations in both species with a higher staining density for human and camel granulocytes, monocytes and a subset of lymphocytes indicates that this antibody detects CD11a in camels.

CD11b is expressed as a hetero-dimer with CD18 (also termed Mac-1 or CR3) mainly on myeloid cells (granulocytes and monocytic cells) (Imhof and Aurrand-Lions, 2004; Nicholson *et al*., 2007) with a lower expression on lymphocytes like NK cells, γδ T cells and a small subset of CD8+ T cells (Fiorentini *et al*., 2001; Graff and Jutila, 2007). The higher staining intensity of human and camel granulocytes and monocytes with the mAb 557743 (clone ICRF44) specific for human CD11b and the weaker staining of lymphocytes indicates that this mAb recognizes CD11b in both species.

CD14 is a co-receptor for bacterial lipopolysaccharides and is mainly expressed on blood monocytes (Hussen *et al*., 2013). Three clones of antibodies specific to human CD14 have been tested for cross-reactivity with camel leukocytes. Only the two mAbs 555398 (clone M5E2) and MCA1568PE (clone Tük4) stained camel monocytes but not granulocytes or lymphocytes, which indicates that these mAbs recognize camel CD14.

Human MHC class II antigen (HLA-DR) is constitutively expressed on professional antigen-presenting cells like dendritic cells, B cells, and monocytes (Abeles *et al*., 2012). The mAb 347403 (clone L243), which stained human monocytes and a population of lymphocytes, induced only a weak staining response in camel monocytes and lymphocytes. It is likely that this mAb has a low affinity to camel MHC-II molecules.

In summary, the present study aimed at providing the field of immunology with new antibodies to camel leukocyte antigens. Although some useful mAbs (about 10 % of studied 52 mAb) have been identified in the present study, which may contribute to fill the gap of available reagents for studying the immune response of camels to infectious pathogens and their response to vaccination, there is a clear need for developing mAbs to more camel leukocyte antigens. Also further cross-reactivity studies using mAbs against leukocyte antigens of animals with close sequence homology to camels like cattle or pigs could be helpful in identifying more cross-reactive antibodies.

Acknowledgements

The cooperation and assistance of the Al Omran slaughter house personal is greatly acknowledged

Conflict of interest

The authors declare that there is no conflict of interest.

References

Abeles, R.D., McPhail, M.J., Sowter, D., Antoniades, C.G., Vergis, N., Vijay, G.K., Xystrakis, E., Khamri, W., Shawcross, D.L., Ma, Y., Wendon, J.A. 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 A 81(10), 823-834.

Al-Mohammed Salem, K., Badi, F.A., Al Haroon, A.I. and Alluwaimi, A.M. 2012. The Cellular Populations of Normal Camel (Camelus dromedaries) Milk. Open J. Vet. Med. 2, 262-265.

Davis, W.C., Davis, J.E. and Hamilton, M.J. 1995. Use of monoclonal antibodies and flow cytometry to cluster and analyze leukocyte differentiation molecules. Methods Mol. Biol. 45, 149-167.

Davis, W.C., Drbal, K., Mosaad, A.E., Elbagory, A.R., Tibary, A., Barrington, G.M., Park, Y.H. and Hamilton, M.J. 2007. Use of flow cytometry to identify monoclonal antibodies that recognize conserved epitopes on orthologous leukocyte differentiation antigens in goats, llamas, and rabbits. Vet. Immunol. Immunopathol. 119(1-2), 123-130.

Davis, W.C. and Hamilton, M.J. 2008. Use of flow cytometry to develop and characterize a set of monoclonal antibodies specific for rabbit leukocyte differentiation molecules. J. Vet. Sci. 9(1), 51-66.

Drbal, K., Angelisova, P., Cerny, J., Pavlistova, D., Cebecauer, M., Novak, P. and Horejsi, V. 2000. Human leukocytes contain a large pool of free forms of CD18. Biochem. Biophys. Res. Commun. 275(2), 295-299.

Duvel, A., Maass, J., Heppelmann, M., Hussen, J., Koy, M., Piechotta, M., Sandra, O., Smith, D.G., Sheldon, I.M., Dieuzy-Labaye, I., Zieger, P. and Schuberth, H.J. 2014. Peripheral blood leukocytes of cows with subclinical endometritis show an altered cellular composition and gene expression. Theriogenology 81(7), 906-917.

Fiorentini, S., Licenziati, S., Alessandri, G., Castelli, F., Caligaris, S., Bonafede, M., Grassi, M., Garrafa, E., Balsari, A., Turano, A. and Caruso, A. 2001. CD11b expression identifies CD8+CD28+ T lymphocytes with phenotype and function of both naive/memory and effector cells. J. Immunol. 166(2), 900-907.

Gerner, W., Talker, S.C., Koinig, H.C., Sedlak, C., Mair, K.H. and Saalmuller, A. 2015. Phenotypic and functional differentiation of porcine alphabeta T cells: current knowledge and available tools. Mol. Immunol. 66(1), 3-13.

Graff, J.C. and Jutila, M.A. 2007. Differential regulation of CD11b on gammadelta T cells and monocytes in response to unripe apple polyphenols. J. Leukoc. Biol. 82(3), 603-607.

Hamers-Casterman, C., Atarhouch, T., Muyldermans, S., Robinson, G., Hamers, C., Songa, E.B., Bendahman, N. and Hamers, R. 1993. Naturally occurring antibodies devoid of light chains. Nature 363(6428), 446-448.

Harris, E.S., McIntyre, T.M., Prescott, S.M. and Zimmerman, G.A. 2000. The leukocyte integrins. J. Biol. Chem. 275(31), 23409-23412.

Hopkins, J., Ross, A. and Dutia, B.M. 1993. Summary of workshop findings of leukocyte antigens in sheep. Vet. Immunol. Immunopathol. 39, 49-59.

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

Hussen, J., Koy, M., Petzl, W. and Schuberth, H.J. 2016. Neutrophil degranulation differentially modulates phenotype and function of bovine monocyte subsets. Innate Immun. 22(2), 124-137.

Imhof, B.A. and Aurrand-Lions, M. 2004. Adhesion mechanisms regulating the migration of monocytes. Nat. Rev. Immunol. 4(6), 432-444.

Lunn, D.P., Holmes, M.A., Antczak, D.F., Agerwal, N., Baker, J., Bendali-Ahcene, S., Blanchard-Channell, M., Byrne, K.M., Cannizzo, K., Davis, W., Hamilton, M.J., Hannant, D., Kondo, T., Kydd, J.H., Monier, M.C., Moore, P.F., O'Neil, T., Schram, B.R., Sheoran, A., Stott, J.L., Sugiura, T. and Vagnoni, K.E. 1998. Report of the Second Equine Leucocyte Antigen Workshop, Squaw valley, California, July 1995. Vet. Immunol. Immunopathol. 62(2), 101-143.

MacHugh, N.D., Bensaid, A., Howard, C.J., Davis, W.C. and Morrison, W.I. 1991. Analysis of the reactivity of anti-bovine CD8 monoclonal antibodies with cloned T cell lines and mouse L-cells transfected with bovine CD8. Vet. Immunol. Immunopathol. 27(1-3), 169-172.

Maecker, H.T., McCoy, J.P. and Nussenblatt, R. 2012. Standardizing immunophenotyping for the Human Immunology Project. Nat. Rev. Immunol. 12(3), 191-200.

Muyldermans, S. 2013. Nanobodies: natural single-domain antibodies. Annu. Rev. Biochem. 82, 775-797.

Naessens, J., Olubayo, R.O., Davis, W.C. and Hopkins, J. 1993. Cross-reactivity of workshop antibodies

with cells from domestic and wild ruminants. Vet. Immunol. Immunopathol. 39(1-3), 283-290.

Nicholson, G.C., Tennant, R.C., Carpenter, D.C., Sarau, H.M., Kon, O.M., Barnes, P.J., Salmon, M., Vessey, R.S., Tal-Singer, R. and Hansel, T.T. 2007. A novel flow cytometric assay of human whole blood neutrophil and monocyte CD11b levels: upregulation by chemokines is related to receptor expression, comparison with neutrophil shape change, and effects of a chemokine receptor (CXCR2) antagonist. Pulm. Pharmacol. Ther. 20(1), 52-59.

Schafer, H. and Burger, R. 2012. Tools for cellular immunology and vaccine research the in the guinea pig: monoclonal antibodies to cell surface antigens and cell lines. Vaccine 30(40), 5804-5811.

van Kooyk, Y. and Figdor, C.G. 2000. Avidity regulation of integrins: the driving force in leukocyte adhesion. Curr. Opin. Cell Biol. 12(5), 542-547.

Zidan, M., Kassem, A. and Pabst, R. 2000a. Megakaryocytes and platelets in the spleen of the dromedary camel (Camelus dromedarius). Anat. Histol. Embryol. 29(4), 221-224.

Zidan, M., Schuberth, H.J. and Pabst, R. 2000b. Immunohistology of the splenic compartments of the one humped camel (Camelus dromedarius). Vet. Immunol. Immunopathol. 74(1-2), 17-29.