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Interaction of Specific Monoclonal Antibodies with Leukocyte Antigens in Camels

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ABSTRACT

The dromedary camel as a livestock species significantly impacts the economy of arid and semi-arid regions worldwide. The identification of cross-reactive antibodies against pivotal immune cell markers acts as a valuable method to investigate the immune system of camels. The aim of the present study was to identify new monoclonal antibodies that react with camel leukocyte subsets using flow cytometry and multicolor immunofluorescence. The expression patterns of the tested antibodies indicated cross-reactivity of the anti-bovine CD9 monoclonal antibody clones LT86A and Hl9a with different binding potential. Although all leukocyte subpopulations stained positively with the CD9 antibodies, monocytes showed the highest CD9 abundance, compared to lymphocytes and granulocytes. No cross-reactivity was identified for the tested monoclonal antibodies against equine CD8a (clone: ETC142BA1), mouse CD3 (clone: CD3-12), human CD3 (clone: T3/2/16A9), human CD206 (clone: MMR), and bovine granulocytes (clone: CH138A). The present study revealed that only camel monocytes showed positive staining with the anti-ovine CD5 mAb (clone ST1), which is in contrast to the human and murine systems. The present findings indicated low homogeneity between camels and other species in the antigenic structure of leukocyte antigens, highlighting the need to develop camel-specific mAbs against the main immune cell markers.

Keywords: Antibodies, Camel, Cell marker, Flow cytometry, Immunity

INTRODUCTION

Monoclonal antibodies (mAbs) play a crucial role in the process of identifying immune cells and monitoring their distribution and mobilization in distinct tissues such as the lungs, the ovary, and the lymph node (Gunnes et al., 2003; Maecker et al., 2012; Hussen et al., 2022; Rivers et al., 2022). In order to avoid the laborious procedure of generating species-specific mAbs, researchers frequently employ cross-reactive mAbs (Farady et al., 2009; Irani et al., 2016; Grandoni et al., 2020; Grandoni et al., 2023; Hussen et al., 2023a; Hussen et al., 2023b). Over the past few years, several studies have been performed to test the cross-reactivity of mAbs against leukocyte antigens sourced from humans or other veterinary animals with their corresponding camel antigens (Hussen et al., 2017; Hussen and Schuberth, 2020; Hussen et al., 2022). These studies have successfully identified cross-reactive mAbs that target significant cell marker antigens. The list of mAbs and the target cluster of differentiation antigen molecules have been recently reviewed (Hussen and Schuberth, 2020). The identification of such mAbs has been employed in several important studies on camel immune cells (Hussen et al., 2020a; Hussen et al., 2020b; Hussen et al., 2021; Hussen and Al-Sukruwah, 2022; Hussen et al., 2022; Hussen et al., 2023a; Hussen et al., 2023b).

Flow cytometry has been demonstrated to be a valuable technique for identifying and characterizing immune cells in body fluids or cell suspensions prepared from tissue homogenates. The relevancy of this technique has been examined in the fields of infection immunity, immune response to vaccination, immunopathology, and tumor research and diagnosis through the determination of the manner in which a population of cells is distributed within tissue homogenates (Koshiol and Lin, 2012; Lyons et al., 2017; Boonyaratanakornkit and Taylor, 2019).

The cell marker CD9 is one of the tetraspanin family with a key role in the essential cellular functions of many immune cells and endothelial cells. This mainly includes intracellular signaling, cell stimulation and proliferation, cell viability, and cell adhesion and migration (Veenbergen and van Spriel, 2011; Rocha-Perugini et al., 2014). CD9 exhibits a broad distribution among cells and tissues and was initially recognized as a marker for lymphohematopoiesis (Rocha-Perugini et al., 2014). Subsequently, it became associated with numerous cellular processes such as motility, proliferation, differentiation, fusion, and adhesion (Wright et al., 2004; Tohami et al., 2004; Pugholm et al., 2016).

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The identification of T cells is usually possible using antibodies to the pan-T cell marker CD3 in combination with the T cell co-receptors CD4 and CD8. Although camel helper T cells can be identified using anti-bovine CD4 antibody, there are no antibodies to camel CD3 or CD8. In addition, the set of available mAbs against camel T cell activation markers is very limited. The transmembrane receptor CD5 is a 67 kDa type 1 T cell surface protein with key regulatory roles in T cell activation and development (Burgueno-Bucio et al., 2019). In humans and mice, CD5 is expressed in thymocytes, mature T cells, and a subset of B cells known as B1a (Li et al., 2019). Although the role of CD5 in lymphocytes has been subjected to extensive investigation, its function in other populations of immune cells remains largely unexplored. In T cells, the primary function of CD5 is to regulate signaling through the T cell receptor (TCR). This is in addition to their role during the development of thymocytes (Axtell et al., 2006). Furthermore, CD5 showed an inhibitory effect on peripheral T cells through interference with the immunological synapse formation (Raman, 2002). Therefore, increased CD5 expression in T cells is associated with a suppressed response to stimulation by antigens (Hawiger et al., 2004). On the other hand, a high level of CD5 expression in T cells contributes to the induction of tolerance and the generation of regulatory T cells (Treg) (Ordonez-Rueda et al., 2009).

The main target of the present study was to test the reactivity of camel leukocyte subsets with some commercially available monoclonal antibodies for leukocyte antigens from other species using flow cytometry and multicolor immunofluorescence. The identification of reactive monoclonal antibodies against new cell surface antigens will pave the way for in-depth exploration of the camel immune system.

MATERIALS AND METHODS

Ethical approval

Animals' ethical approval was obtained from the Ethics Committee of King Faisal University, Saudi Arabia, with an approval number (KFU-REC-2021- DEC -EA000326).

Blood samples

Blood samples (10 mL blood) were collected from five male dromedary camels of the Al-Majahim breed (between 8 and 13 years with an average weight of 468 ± 46 kg). The animals were selected from the camels admitted to Al-Omran Slaughterhouse in Al-Ahsa Region in Saudi Arabia. All animals were apparently healthy based on the clinical examination (performed by a veterinarian) for any symptoms such as mastitis, metritis, diarrhea, and respiratory diseases. The samples were collected from the jugular vein into blood collection tubes with the anticoagulation agent EDTA (Becton Dickinson, Heidelberg, Germany) and transported to the laboratory within one hour.

Purification of camel leukocytes

Peripheral blood leukocytes were separated as previously described (Hussen, 2021). Briefly, 5 mL camel blood was diluted 1:2 in cold phaosphate buffered saline (PBS) and centrifuged for 20 minutes at 3000 rpm and 10 ºC. After removing the supernatant plasma, the remaining pellet of leukocytes and red blood cells was suspended and incubated for 20 seconds with 10 mL aqua dist. Followed by the addition of 10 mL of 2-times concentrated PBS. This lysis step was repeated 2-3 times (centrifugation at 1550 rpm, 1100 rpm, and 100 ×g for 10 min at 10 ºC) until complete removal of RBCs. Finally, the cell pellet was suspended in PBS and adjusted to 5×10^6 cell/mL. Cell viability (always more than 95%) was evaluated by flow cytometry after adding propidium iodide (2 µg/mL) to the cells.

Membrane immunofluorescence and flow cytometry

Separated blood leukocytes were labeled with monoclonal antibodies (mAbs) to selected leukocyte antigens and analyzed by flow cytometry (Eger et al., 2015; Hussen et al., 2013). Separated cells (5×10^5 cell/well) were incubated in the wells of a 96-well plate with mAbs to cluster of differentiation CD9, CD5, CD3, CD206, CD46, CD163, and the granulocyte marker CH138A (Table 1). After incubation at 4°C for 15 minutes, cold PBS was added to the cells (150 µL per well) to wash out unbound antibodies. After that, cells were stained with secondary fluorochromes-labeled anti-mouse antibodies (Invitrogen) or with isotype controls (Becton Dickinson Biosciences). After washing, cells were analyzed on flow cytometry (Becton Dickinson Accuri C6 flow cytometer San Jose, California, USA). At least 100000 cells were measured and analyzed with C-Flow.

Statistical analysis

Data analysis was performed using the flow cytometric software C-Flow (Becton, Dickinson; Accuri C6 Software 1.0.264.21 BD, USA). Means and standard error of the mean (SEM) were calculated using the column statistic function of the Prism software (GraphPad version 5; California, USA). Differences between means were tested with a t-test (for repeated measures), with p value of less than 0.05 considered significant.

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Table 1. List of monoclonal antibodies involved in the study to test their reactivity with camel antigens

Antigen	Labeling	Antibody clone	Target species	Source
CD9	-	LT86A	Bovine	Kingfisher, USA
CD9	PE	Hl9a (V P018)	Bovine	BioLegend, UK
CD8a	-	ETC142BA1	Equine	Kingfisher, USA
CD46	FITC	MEM-258	Bovine	Biorad, UK
CD3	FITC	CD3-12	Mouse	Abcam, USA
CD3	-	T3/2/16A9	Human	Mybiosource, USA
CD206	PE	MMR	Human	BioLegend, USA
Granulocyte	-	CH138A	Bovine	Kingfisher, USA
CD5	-	ST1	Ovine	Kingfisher, USA

CD: Cluster of differentiation, PE: Phycoerythrin, FITC: Fluorescein isothiocyanate.

RESULTS AND DISCUSSION

Monoclonal antibodies to cell surface antigens are valuable tools for characterizing immune cells and monitoring changes in immune cell phenotype during infection or vaccination (Maecker et al., 2012). Although some subsets of camel immune cells can be identified using cross-reactive antibodies (Hussen et al., 2017), comprehensive immunophenotyping of camel immune cells requires the identification of other monoclonal antibodies to extend the toolbox for the characterization of important cell populations and subpopulations.

The cell marker CD9 is one of the tetraspanin family with a key role in the essential cellular functions of many immune cells and endothelial cells (Kinashi, 2005; Veenbergen and van Spriel, 2011; Rocha-Perugini et al., 2014). In the present study, two clones of antibodies (Hl9a and LT86A) against bovine CD9 showed reactivity with camel leukocytes (Figure 1A-B). Although both clones showed the same expression pattern with significantly higher ($p < 0.05$) expression on camel monocytes compared to lymphocytes and granulocytes, the staining intensity with the Hl9a clone was significantly (6 times) higher than the LT86A clone ($p < 0.05$). A possible explanation for this difference is the magnification of the fluorescence signal due to the use of an indirect membrane immunofluorescence test to detect the unlabeled Hl9a mAb, while the LT86A mAb was directly labeled with phycoerythrin (PE, Figure 1A-D). The current results confirm the previous reports about the wide distribution of CD9 on human leukocyte subsets, including B cells, helper CD4+ T cells, cytotoxic CD8+ T cells, natural killer cells, and myeloid cells (Wright et al., 2004; Tohami et al., 2004; Pugholm et al., 2016) and indicates similar expression pattern of CD9 on camel and human leukocytes. Given its key role in several functions of different immune cells, the identification of two monoclonal antibodies with cross-reactivity against camel CD9 will enable conducting functional studies to uncover its role in the camel immune system.

The characterization of T cell subpopulations is usually achieved by the combined staining with mAbs to the pan-T cell marker CD3 and the T cell co-receptors CD4 and CD4 to identify helper and cytotoxic T cells, respectively. In the present study, the anti-mouse CD3 mAbs clone CD3-12 and the anti-human CD3 mAb clone (T3/2/16A9) did not show any reactivity with camel lymphocytes, indicating no cross-reactivity with the corresponding camel CD3 antigen (Figure 2). Similarly, an anti-equine CD8alpha mAb (clone: ETC142BA1) did not label any cell population within camel lymphocytes (Figure 2).

Currently, the identification of camel myeloid cell subsets depends on using mAbs to the pan-myeloid marker CD172a, the monocyte markers CD14 and CD163 in combination with the major histocompatibility complex (MHC) molecules II. Furthermore, camel leukocytes did not show reactivity to monoclonal antibodies to the bovine pan-granulocyte marker (clone: CH138A), the human macrophage marker CD206 (clone: MMR, Figure 2), and the bovine CD46 antigen (MEM-258).

The transmembrane receptor CD5 is a 67 kDa type 1 T cell surface protein with key regulatory roles in T cell activation and development (Raman, 2002). In humans and mice, CD5 expression has been considered limited to cells of the lymphoid lineage, mainly on thymocytes, T, and B lymphocytes (Li et al., 2019). Recently, CD5 was detected in dendritic cells from human blood (Wood and Freudenthal, 1992; Li et al., 2019; He et al., 2023) and monocyte-derived dendritic cells and macrophages (Takahashi et al., 1998; Helft et al., 2015). In the present study, camel lymphocytes showed no reactivity with the anti-ovine CD5 mAb (clone: ST1). On the other hand, the antibody showed positive staining for camel monocytes that were identified by their CD163 expression. This is in contrast to the negative staining of monocytes to the isotype control antibodies (Figure 3A-E). Whether the expression of CD5 on camel monocytes represents a camel-specific expression pattern of CD5 or whether the antibody nonspecifically recognizes an epitope on camel monocytes could not be answered based on the data of the current study. To clarify this, blocking FC receptors on camel monocytes and comparative staining of camel and human leukocytes with the CD5 antibody are required.

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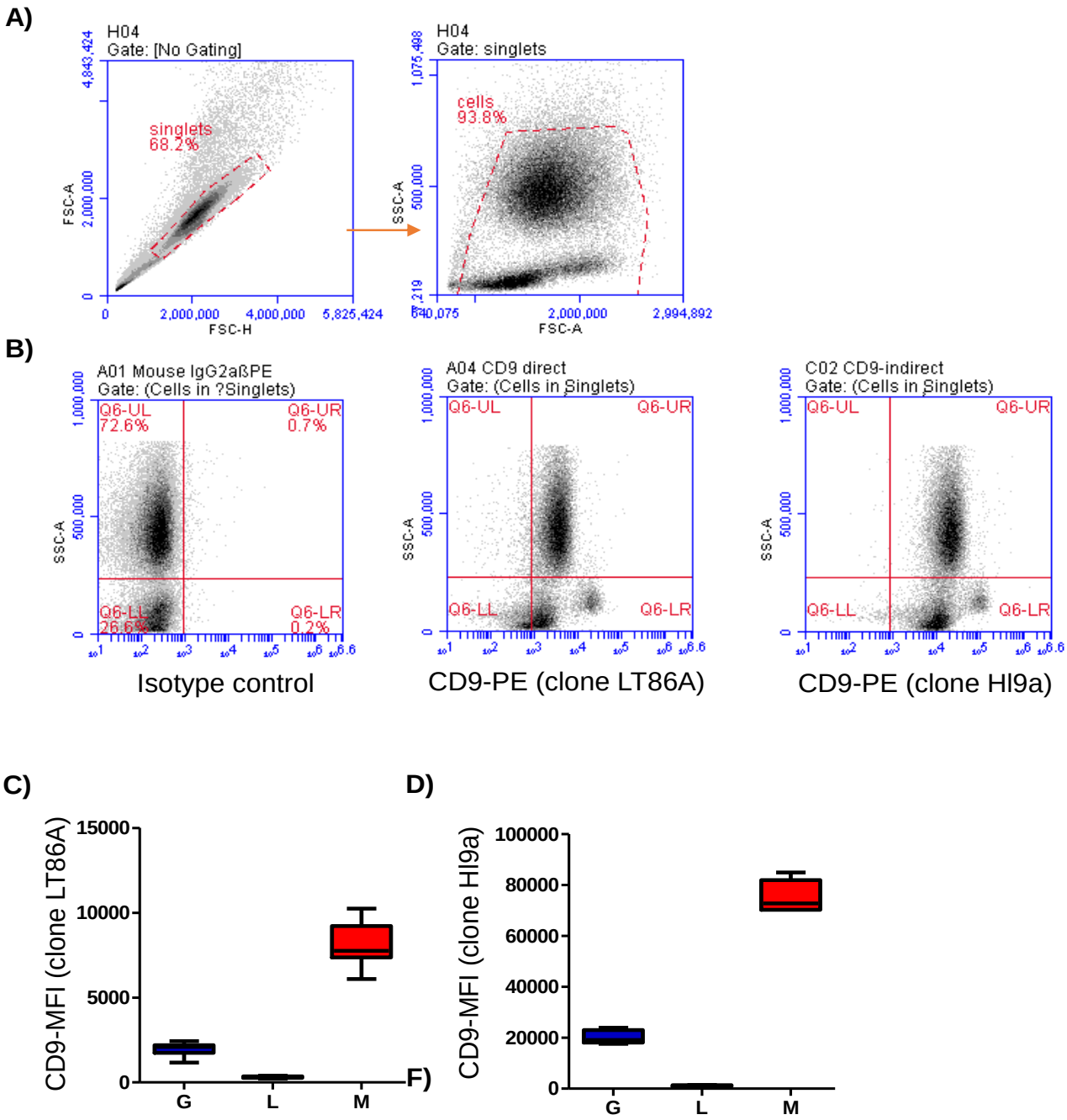

Figure 1. Reaction of camel immune cells toward mAbs against CD9 antigen. Cell duplicates were excluded from the analysis based on forward scatter (FSC)-H/FSC-A signals, and leukocytes were gated in a side scatter (SSC)-A/FSC-A dot plot to exclude cell debris (**A**). Staining of leukocytes with isotype control antibody or with CD9-specific antibodies of the clone LT86A or Hl9a (**B**). Mean fluorescence intensity (MFI) values were presented for granulocytes, lymphocytes, and monocytes (n= 5 animals) were presented for the clone LT86A (**C**) or Hl9a (**D**). The letters G, L, and M indicate granulocytes, lymphocytes, and monocytes, respectively.

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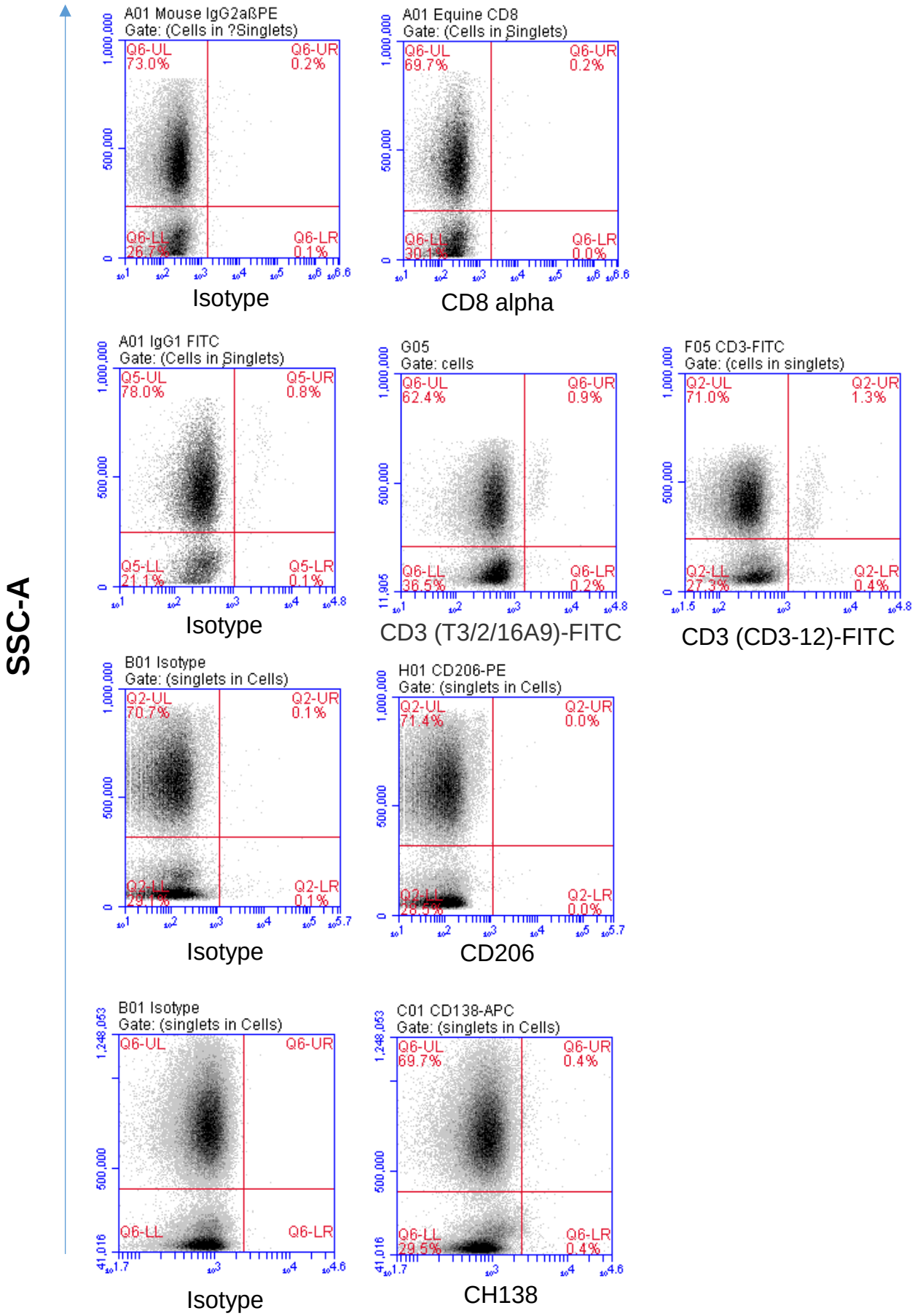

Figure 2. Staining of camel leukocytes with monoclonal antibodies to selected leukocyte antigens from other species or with isotype antibody controls. Camel leukocytes were labeled with indicated antibodies and analyzed on the accuri cytometer. Representative side scatter against staining (fluorescence intensity) dot plots of five separate experiments.

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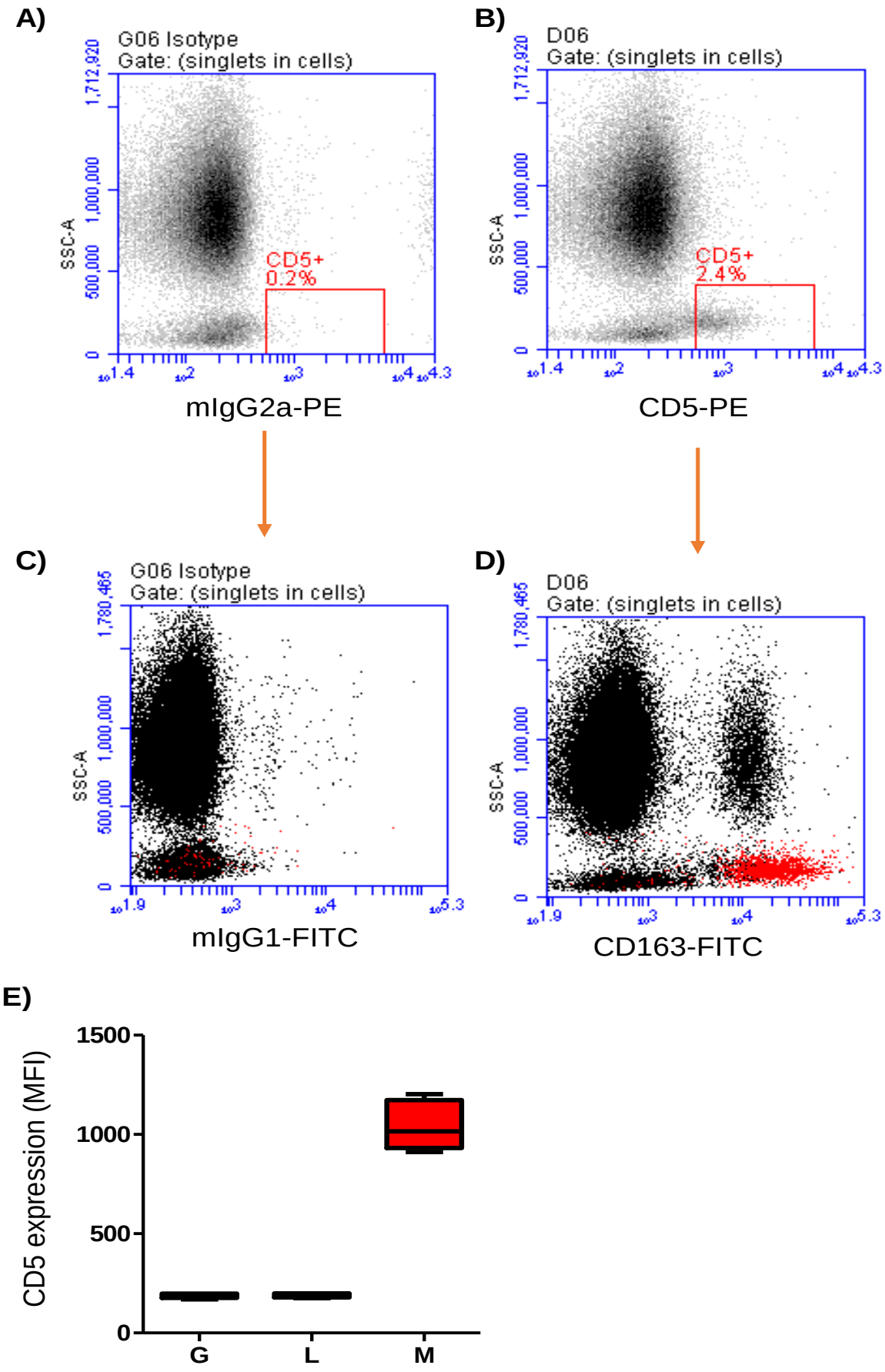

Figure 3. Reactivity of camel leukocytes with isotype control (**A**) or specific antibodies against ovine CD5 (**B**). A gate was set on CD5+ cells (red gate), and positive cells were marked in red in a separate dot plot of mouse IgG1 isotype control (**C**) or CD163 (**D**), indicating the co-expression of CD163 and CD5 on monocytes. **E**: Mean fluorescence intensity values of CD5 expression were presented for camel leukocyte subsets. The letters G, L, and M indicate granulocytes, lymphocytes, and monocytes, respectively.

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CONCLUSION

The present study tested the reactivity of camel leukocyte subsets with some commercially available monoclonal antibodies for leukocyte antigens from other species. The expression patterns of the tested antibodies indicate cross-reactivity of the anti-bovine CD9 monoclonal antibody clones LT86A and Hl9a with different binding potential. In contrast, no cross-reactivity was identified for the anti-equine CD8a mAb (ETC142BA1), anti-mouse CD3 mAb (CD3-12), anti-human CD3 mAb (T3/2/16A9), anti-human CD206 mAb (MMR), anti-bovine granulocytes mAb (CH138A). Only camel monocytes showed positive staining with the anti-ovine CD5 mAb (clone ST1), in contrast to the human and murine systems. The present study indicated low homogeneity between camels and other species in the antigenic structure of leukocyte antigens. It strengthened the need to develop camel-specific mAbs against the main immune cell markers. Further studies may focus on using the identified monoclonal antibodies for phenotypic and functional studies on the camel immune system.

DECLARATIONS

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Availability of data and materials

The datasets generated during the current study are available from the corresponding author upon reasonable request.

Authors' contributions

Mohammed Ameer Alalai did sample collection and manuscript revision; Mayyadah Abdullah Alkuwayti did supervision and manuscript preparation; Noof Abdulrahman Alrabiah did manuscript preparation and revision. Jamal Hussen did the analysis, funding acquisition, and writing of the manuscript. All authors read and approved the final version of the manuscript.

Competing interests

There are no competing interests to disclose.

Ethical consideration

Animals ethical approval was obtained from the Ethics Committee of King Faisal University, Saudi Arabia, with an approval number (KFU-REC-2021- DEC -EA000326). Ethical issues, such as data fabrication, double publication, and submission, redundancy, plagiarism, consent to publish, and misconduct, have been checked by all the authors before publication in this journal.

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