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THE IMMUNOPHENOTYPE OF BLOOD MONONUCLEAR CELLS IN THREE AGE GROUPS OF DROMEDARY CAMELS (*Camelus dromedarius*)

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ABSTRACT

Age related changes in the immune system were described for different humoral and cellular immune components in man and animal. Studies on the development of the cellular immune system in camels are limited. Therefore, the present study compared the three age groups of camels regarding the immunophenotype of mononuclear cells in their blood. Thirty-four dromedary camels were divided, based on their age, into three groups (G) with camels in G1 aged 3 - 11 month, camels in G2 aged 2 - 5 years, and G3 camels aged 6 - 10 years. The immunophenotype of mononuclear cells was analysed by flow cytometry. The results revealed higher percentage of lymphocytes in G1 and G2 than G3, while lymphocyte absolute numbers were only higher in G1 than the other two groups. Within lymphocytes, the absolute numbers of WC1+ T cells and B cells were highest in G1 compared to the other two groups, while the absolute numbers of CD4+ T cells did not show significant differences between the groups. In addition, the reduced abundance of CD11ahigh and CD44high lymphocytes together with the reduced expression of CD9 on lymphocytes and MHC II on monocytes indicate the reduced maturity of the camel immune system during the first year of life. In conclusion, the present study identified significant age-related changes in the immunophenotype of mononuclear cells in camel blood. The changes are characterised by a decrease in the number of lymphocyte, gd T cells, and B cells. In addition, age was associated with an expansion in activated lymphocytes and monocytes in camel blood.

Key words: Age, dromedary camel, flow cytometry, lymphocytes, monocytes

Blood mononuclear cells, including cells from the lymphoid (lymphocytes) and myeloid (monocytes) lineage with key roles in innate and adaptive immune functions, are easily accessible tool for studying the immune system. Immunophenotyping of mononuclear cells by flow cytometry has been intensively used for the identification of changes in the distribution of cell subsets in different physiological and pathological conditions (Johnson *et al,* 2022; Li *et al,* 2022; Heubeck *et al,* 2023).

Expressed on all leukocytes, CD11a dimerizes with CD18 to form the adhesion molecule lymphocyte function antigen-1 (LFA-1) (Roos and Law, 2001; van de Vijver *et al,* 2012). The lymphocyte homing receptor CD44 plays an essential role in lymphocyte adhesion and migration (Schumann *et al,* 2015). Both CD11a and CD44 are prominent activation markers of lymphocytes (McDermott and Varga 2011; Schumann *et al,* 2015). The tetraspanin CD9 is widely expressed molecule (expressed by several lymphoid and myeloid cells as well as by endothelial cells). In leukocytes, CD9 has been found involved in many cellular activities, including proliferation, activation, adhesion and migration (Reyes *et al,* 2018). Major histocompatibility (MHC) class II molecules are expressed on antigen presenting cells, including monocytes and B cells in blood as well as macrophages and dendritic cells in tissue (Holling *et al,* 2004; Zheng *et al,* 2022). MHC II are antigen receptors that present peptide antigens to T helper cells (Abeles *et al,* 2012).

Age-related changes in the phenotype and function of several immune cells were reported for several species (Hussein *et al,* 1992; Hulstaert *et al,* 1994; Ayoub and Yang 1996; Yan *et al,* 2010; Lin *et al,* 2016). Compared to other livestock, studies on the cellular immune system of camel are still very limited (Zidan *et al,* 2000a; Zidan *et al,* 2000b). The present study compared the distribution and activation status of cellular subsets within blood mononuclear cells in three age groups of camels.

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Materials and Methods

2.1 Animals and blood sampling

Thirty-four dromedary camels (*Camelus dromedarius*) divided into three age groups were involved in the current study. The first group (G1) contained 10 camels aged between 3 and 11 month (mean ± SEM: 7.6 ± 0.7 month); the age group 2 (G2) contained 5 camels aged between 2 and 5 years (3.2 ± 0.5 year); the age group 3 (G3) contained 19 camels aged between 6 and 10 years (7.8 ± 0.3 years). The camels were kept in different private farms in Al-Ahsa region in Saudi Arabia. Blood was obtained by venipuncture of the vena jugularis externa into vacutainer tubes containing EDTA (Becton Dickinson, Heidelberg, Germany).

2.2 Separation of whole leukocytes

Separation of whole camel leukocytes was performed by hypotonic lysis of erythrocytes. For this, 2 mL blood was incubated in 5 mL distilled water for 20 sec and 5 mL of 2x PBS was added to restore tonicity. After centrifugation at 1000 xg for 10 min, the erythrolysis was repeated twice until complete removal of red blood cells. Separated cells were finally suspended in PBS containing bovine serum albumin at 2 x 10^6 cells/ml.

2.3 Cell labeling and flow cytometry

Camel leukocytes (2 x 10^5 / well of 96 well plate) were incubated with monoclonal antibodies specific for CD4, WC1, CD9, CD11a, CD44, and MHC-II molecules in PBS containing bovine serum albumin (Hussen, 2021). After 15 minutes incubation at 4°C, cells labelled with primary antibodies were washed twice and incubated with secondary antibodies to mouse IgG1, IgG2a, and IgM. After final wash, the cells were analysed on the flow cytometer (Accuri C6; BD).

Statistical Analyses

Statistical analysis was performed with Prism (GraphPad). Results are presented as means ± S.E. of the mean (SEM). Differences between means were tested with one-factorial analysis of variance (ANOVA) and Bonferroni's correction for normally distributed data. Results were considered significant at a p-value of less than 0.05.

Results and Discussion

The percentage of lymphocytes (Fig 1A) was significantly ($p < 0.05$) higher in G1 (33.1 ± 1.4 % of leukocytes) and G2 (24.2 ± 1.6 % of leukocytes) than G3 (19.3 ± 1.5 % of leukocytes), while lymphocyte absolute numbers (Fig 1B) were only elevated ($p < 0.05$) in G1 (1690 ± 199 cell/µL blood) than G 2 (949 ± 153 cell/µL blood) and G 3 (1116 ± 97 cell/µL blood). Neither the percentages nor the absolute numbers of monocytes differed ($p > 0.05$) between the three groups (Fig 1A and B). The observed higher percentages and numbers of lymphocytes in the younger age group is in line with the physiological age-related lymphocytosis reported in camels (Hussein *et al,* 1992; Gaashan *et al,* 2020). In a previous study, highest lymphocyte numbers in one-month aged camel calves followed by age-associated decrease was reported (Hussein *et al,* 1992).

The estimation of the abundance of subpopulations within camel lymphocytes revealed significant age-related changes. The percentage of CD4+ T cells was higher in G2 (18.1 ± 3.6 % of lymphocytes) and G3 (19.1 ± 1.2 % of lymphocytes) than G1 (11.8 ± 0.8 % of lymphocytes), the difference was, however, only significant ($p < 0.05$) between G1 and G3 (Fig 2A). In contrast to this, the percentage of WC1+ T cells was significantly ($p < 0.05$) higher in G1 (22.7 ± 1.8 % of lymphocytes) and G2 (9.6 ± 2.6 % of lymphocytes) than G3 (3.9 ± 0.4 % of lymphocytes). The fraction of B cells did not show significant differences between the three groups. The absolute numbers of WC1+ T cells were highest in G1 (383 ± 56 cell/µL) compared to the G2 (97 ± 30 cell/µL) and G3 (41 ± 5 cell/µL), while the absolute numbers of CD4+ T cells did not show significant differences between the groups. For B cells absolute number, a significantly higher number was found in the G1 (426 ± 54 cell/µL) than G2 (254 ± 69 cell/µL) and G3 (227 ± 30 cell/µL).

The observed lymphocyte composition confirms the previously reported dominance of γδ T cells over other blood lymphocytes in camel calves during the first year of life (Hussen, 2018; Hussen and Schuberth, 2020). The results also indicated rapid change in γδ T cell frequency with a rapid decrease of their percentages and numbers in the G2 camels aged 2-5 years. Age-related changes in B cell distribution and populations were described in different species (Blanco *et al,* 2018). Early life increase in B cells and subsequent decrease in B cell numbers was described previously for human B cells (Rodriguez-Zhurbenko *et al,* 2019).

The percentage of CD11a^{high} lymphocytes (Fig 3A) was significantly higher in G3 (25.4 ± 2.6% of lymphocytes) than G1 (10.1 ± 1.1% of lymphocytes) and G2 (14.2 ± 1.0% of lymphocytes). For the fraction of CD44a^{high} lymphocytes (Fig 3A),

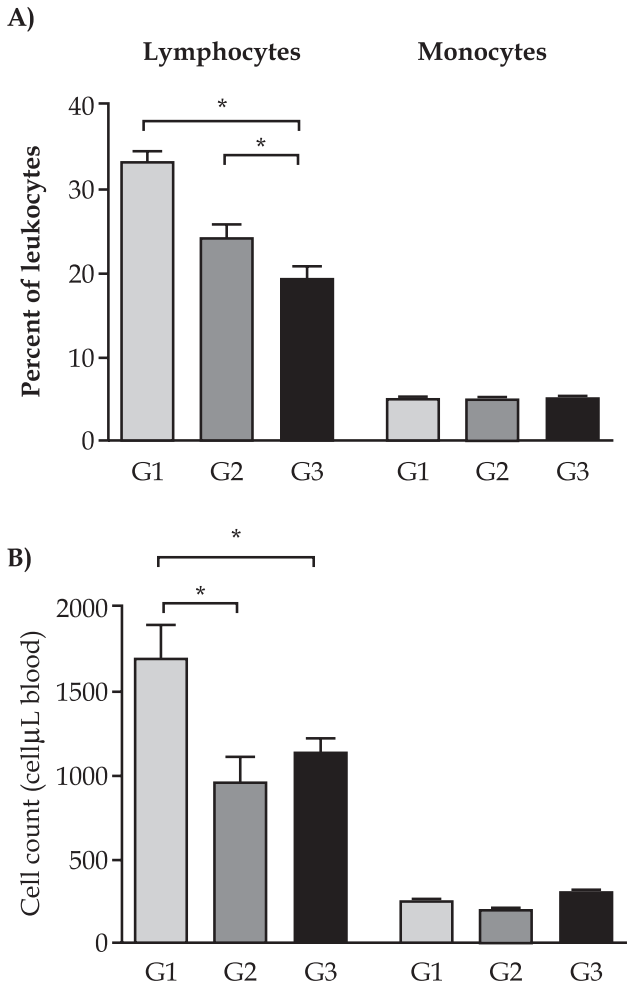

Fig 1. Impact of age on the frequency of camel mononuclear cells. A) The percentage of lymphocytes and monocytes were calculated after flow cytometric identification of the cells based on their forward and side scatter properties. B) The absolute numbers of lymphocytes and monocytes were calculated by multiplication of their percentages by the absolute number of leukocytes counted by light microscopy and Turk solution. * indicates significant ($p < 0.05$; One-Way ANOVA).

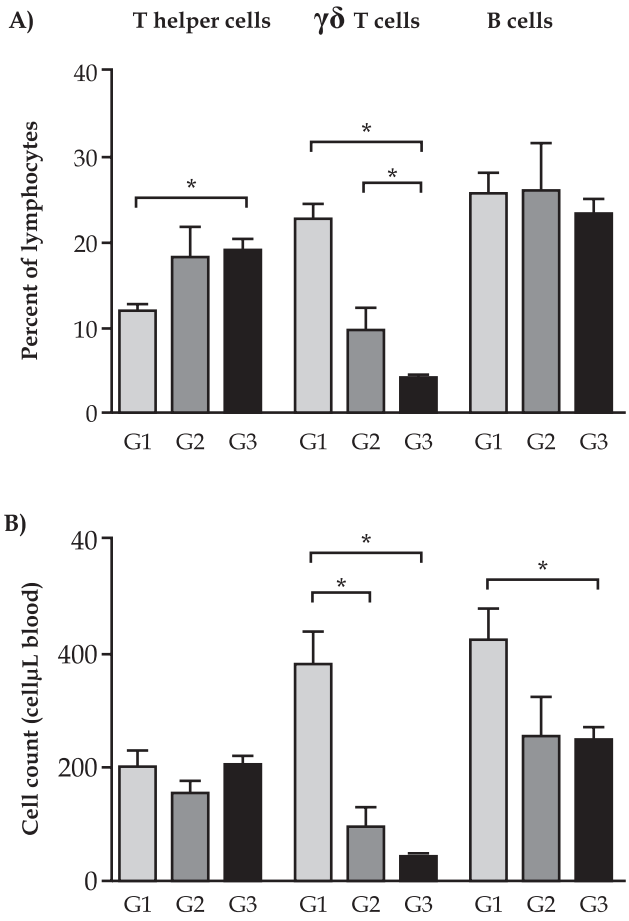

Fig 2. Age-related changes in the frequency of lymphocyte subsets in blood. A) Percentage of helper T cells, gd T cells and B cells were calculated after labeling leukocytes with antibodies to the markers CD4, WC1, and MHC II, respectively. B) The absolute numbers of cell subsets were calculated by multiplication of their percentages by the absolute number of lymphocytes. * indicates significant ($p < 0.05$; One-Way ANOVA).

a higher percentage was observed in G2 (29.9 ± 4.9% of lymphocytes) and G3 (31.2 ± 2.8% of lymphocytes) than in G1 (18.4 ± 1.9% of lymphocytes). The expression density (mean fluorescence intensity; MFI) of CD9 was higher on lymphocytes from G2 (4885 ± 607) and G3 (4536 ± 664) than G1 (2980 ± 320). The difference was however, only significant between G1 and G2 (Fig 3B).

In addition to many other cell markers, CD11a (also known as lymphocyte activator antigen 1) and CD44 are two activation marker of lymphocytes with effector lymphocytes expressing high levels of these molecules (Azeredo *et al,* 2006; Schumann *et al,* 2015). The reduced abundance of lymphocytes expressing high levels of both molecules in the G1 confirms the reduced maturity of the camel immune system during the first year of life. This also confirmed by the reduced abundance of the tetraspanin CD9, an other activation marker highly expressed on activated lymphocytes (Reyes *et al,* 2018). In addition, the early increase in the frequency of $CD44^{high}$ lymphocytes (in G2) than $CD11^{high}$ lymphocytes (in G3) indicates that these molecules identify different cell populations within lymphocytes.

Major histocompatibility complex (MCH) class II molecules are antigen receptors responsible for presenting antigens to the adaptive immune system (Holling *et al,* 2004). Their expression is also considered indicative of monocyte activation status (Hussen *et al,* 2013). In the present study, the

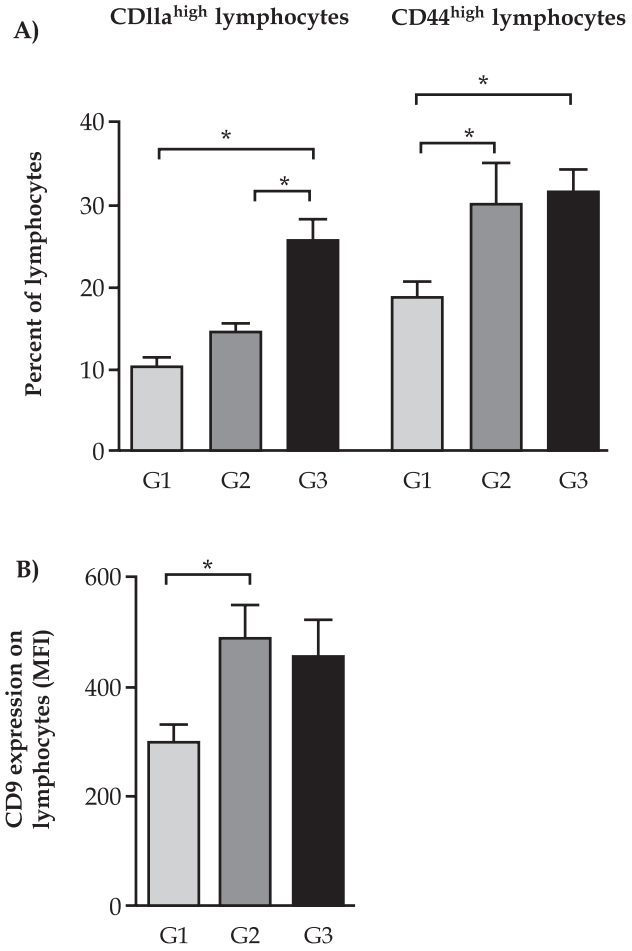

Fig 3. Age-related changes in the percentage of activated lymphocytes. A) Percentage of $CD11a^{high}$ and $CD44^{high}$ lymphocytes were calculated after labeling leukocytes with antibodies to the activation markers CD11a and CD44. B) The abundance of CD9 molecules on lymphocytes. Leukocytes were labeled with antibodies to CD9 molecules and analysed by flow cytometry. The abundance of CD9 molecules on lymphocytes was calculated as mean fluorescence intensity (MFI) and presented for the three groups. * indicates significant ($p < 0.05$; One-Way ANOVA).

abundance (MFI) of MHC II was higher on monocytes from G2 (7529 ± 520) and G3 (6899 ± 395) than G1 (5314 ± 589). However, the difference was only significant between G2 and G1 (Fig 4). These data also supports the reduced maturity of monocytes in camel calves during the first life of age with improved maturity starting with the second year of age.

Ethical approval

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of King Faisal University, Saudi Arabia (KFU-REC-2024-JUN-ETHICS1843).

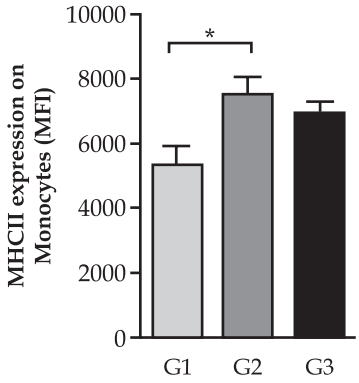

Fig 4. Abundance of MHC II molecules on monocytes. Leukocytes were labeled with antibodies to MHC II molecules and analysed by flow cytometry. The abundance of MHC II molecules on monocytes was calculated as mean fluorescence intensity (MFI) and presented for the three groups. * indicates significant ($p < 0.05$; One-Way ANOVA).

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Conflict of interest:

None.

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